Title: Increase in agricultural triazole fungicide use in the United States, 1992–2016, and possible implications for the development of antifungal-resistant fungi causing human disease

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Abstract

The fungus *Aspergillus fumigatus* is the leading cause of invasive mold infections, which cause severe disease and death in immunocompromised people. Use of triazole antifungal medications in recent decades has improved patient survival; however, triazole-resistant infections have become common in parts of Europe and are emerging in the United States. Triazoles are also a class of fungicides used in plant agriculture, and certain triazole-resistant *A. fumigatus* strains found causing disease in humans have been linked to environmental fungicide use. We examined U.S. temporal and geographic trends in use of triazole fungicides using U.S. Geological Survey agricultural pesticide use estimates. Overall tonnage of triazole fungicide use nationwide changed little during 1992–2005 but increased >4-fold during 2006–2016 to 2.9 million kg in 2016. Triazole fungicide use had occurred mostly in orchards and grapes, wheat, and other crops in 1992–2005, but recent increases in use have occurred primarily in wheat, corn, soybeans, and other crops, particularly in Midwest and Southeast states. Given chemical similarities between triazole fungicides and triazole antifungal drugs used in human medicine, increased monitoring for environmental and clinical triazole resistance in *A. fumigatus* is needed, as well as strategies to mitigate development and spread of resistance.

Background

Invasive aspergillosis is a severe and frequently fatal fungal disease (mortality rate 25%–59%) that most commonly affects people who are immunocompromised (e.g., because of transplant or malignancy) or have structural lung disease (e.g., chronic obstructive pulmonary disease (COPD)) [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"4HI19VF7","properties":{"formattedCitation":"(Kontoyiannis et al. 2010; Pappas et al. 2010; Steinbach et al. 2012)","plainCitation":"(Kontoyiannis et al. 2010; Pappas et al. 2010; Steinbach et al. 2012)","noteIndex":0},"citationItems":[{"id":1794,"uris":["http://zotero.org/users/local/opHgB1Dy/items/PU4NDP8L"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/PU4NDP8L"],"itemData":{"id":1794,"type":"article-journal","abstract":"BACKGROUND: The incidence and epidemiology of invasive fungal infections (IFIs), a leading cause of death among hematopoeitic stem cell transplant (HSCT) recipients, are derived mainly from single-institution retrospective studies.\nMETHODS: The Transplant Associated Infections Surveillance Network, a network of 23 US transplant centers, prospectively enrolled HSCT recipients with proven and probable IFIs occurring between March 2001 and March 2006. We collected denominator data on all HSCTs preformed at each site and clinical, diagnostic, and outcome information for each IFI case. To estimate trends in IFI, we calculated the 12-month cumulative incidence among 9 sequential subcohorts.\nRESULTS: We identified 983 IFIs among 875 HSCT recipients. The median age of the patients was 49 years; 60% were male. Invasive aspergillosis (43%), invasive candidiasis (28%), and zygomycosis (8%) were the most common IFIs. Fifty-nine percent and 61% of IFIs were recognized within 60 days of neutropenia and graft-versus-host disease, respectively. Median onset of candidiasis and aspergillosis after HSCT was 61 days and 99 days, respectively. Within a cohort of 16,200 HSCT recipients who received their first transplants between March 2001 and September 2005 and were followed up through March 2006, we identified 718 IFIs in 639 persons. Twelve-month cumulative incidences, based on the first IFI, were 7.7 cases per 100 transplants for matched unrelated allogeneic, 8.1 cases per 100 transplants for mismatched-related allogeneic, 5.8 cases per 100 transplants for matched-related allogeneic, and 1.2 cases per 100 transplants for autologous HSCT.\nCONCLUSIONS: In this national prospective surveillance study of IFIs in HSCT recipients, the cumulative incidence was highest for aspergillosis, followed by candidiasis. Understanding the epidemiologic trends and burden of IFIs may lead to improved management strategies and study design.","container-title":"Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America","DOI":"10.1086/651263","ISSN":"1537-6591","issue":"8","journalAbbreviation":"Clin. Infect. Dis.","language":"eng","note":"PMID: 20218877","page":"1091-1100","source":"PubMed","title":"Prospective surveillance for invasive fungal infections in hematopoietic stem cell transplant recipients, 2001-2006: overview of the Transplant-Associated Infection Surveillance Network (TRANSNET) Database","title-short":"Prospective surveillance for invasive fungal infections in hematopoietic stem cell transplant recipients, 2001-2006","volume":"50","author":[{"family":"Kontoyiannis","given":"Dimitrios P."},{"family":"Marr","given":"Kieren A."},{"family":"Park","given":"Benjamin J."},{"family":"Alexander","given":"Barbara D."},{"family":"Anaissie","given":"Elias J."},{"family":"Walsh","given":"Thomas J."},{"family":"Ito","given":"James"},{"family":"Andes","given":"David R."},{"family":"Baddley","given":"John W."},{"family":"Brown","given":"Janice M."},{"family":"Brumble","given":"Lisa M."},{"family":"Freifeld","given":"Alison G."},{"family":"Hadley","given":"Susan"},{"family":"Herwaldt","given":"Loreen A."},{"family":"Kauffman","given":"Carol A."},{"family":"Knapp","given":"Katherine"},{"family":"Lyon","given":"G. Marshall"},{"family":"Morrison","given":"Vicki

A."},{"family":"Papanicolaou","given":"Genovefa"},{"family":"Patterson","given":"Thomas F."},{"family":"Perl","given":"Trish M."},{"family":"Schuster","given":"Mindy G."},{"family":"Walker","given":"Randall"},{"family":"Wannemuehler","given":"Kathleen A."},{"family":"Wingard","given":"John R."},{"family":"Chiller","given":"Tom M."},{"family":"Pappas","given":"Peter G."}],"issued":{"date-parts":[["2010",4,15]]}}},{"id":66,"uris":["http://zotero.org/users/local/opHgB1Dy/items/L8CME5VQ"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/L8CME5VQ"],"itemData":{"id":66,"type":"article-journal","abstract":"Background. Invasive fungal infections (IFIs) are a major cause of morbidity and mortality among organ transplant recipients. Multicenter prospective surveillance data to determine disease burden and secular trends are lacking.Methods. The Transplant-Associated Infection Surveillance Network (TRANSNET) is a consortium of 23 US transplant centers, including 15 that contributed to the organ transplant recipient dataset. We prospectively identified IFIs among organ transplant recipients from March, 2001 through March, 2006 at these sites. To explore trends, we calculated the 12-month cumulative incidence among 9 sequential cohorts.Results. During the surveillance period, 1208 IFIs were identified among 1063 organ transplant recipients. The most common IFIs were invasive candidiasis (53%), invasive aspergillosis (19%), cryptococcosis (8%), non-Aspergillus molds (8%), endemic fungi (5%), and zygomycosis (2%). Median time to onset of candidiasis, aspergillosis, and cryptococcosis was 103, 184, and 575 days, respectively. Among a cohort of 16,808 patients who underwent transplantation between March 2001 and September 2005 and were followed through March 2006, a total of 729 IFIs were reported among 633 persons. One-year cumulative incidences of the first IFI were 11.6%, 8.6%, 4.7%, 4.0%, 3.4%, and 1.3% for small bowel, lung, liver, heart, pancreas, and kidney transplant recipients, respectively. One-year incidence was highest for invasive candidiasis (1.95%) and aspergillosis (0.65%). Trend analysis showed a slight increase in cumulative incidence from 2002 to 2005.Conclusions. We detected a slight increase in IFIs during the surveillance period. These data provide important insights into the timing and incidence of IFIs among organ transplant recipients, which can help to focus effective prevention and treatment strategies.","container-title":"Clinical Infectious Diseases","DOI":"10.1086/651262","ISSN":"1058-4838","issue":"8","journalAbbreviation":"Clin Infect Dis","language":"en","page":"1101-1111","source":"academic.oup.com","title":"Invasive Fungal Infections among Organ Transplant Recipients: Results of the Transplant-Associated Infection Surveillance Network (TRANSNET)","title-short":"Invasive Fungal Infections among Organ Transplant Recipients","volume":"50","author":[{"family":"Pappas","given":"Peter G."},{"family":"Alexander","given":"Barbara D."},{"family":"Andes","given":"David R."},{"family":"Hadley","given":"Susan"},{"family":"Kauffman","given":"Carol A."},{"family":"Freifeld","given":"Alison"},{"family":"Anaissie","given":"Elias J."},{"family":"Brumble","given":"Lisa M."},{"family":"Herwaldt","given":"Loreen"},{"family":"Ito","given":"James"},{"family":"Kontoyiannis","given":"Dimitrios P."},{"family":"Lyon","given":"G. Marshall"},{"family":"Marr","given":"Kieren A."},{"family":"Morrison","given":"Vicki A."},{"family":"Park","given":"Benjamin J."},{"family":"Patterson","given":"Thomas F."},{"family":"Perl","given":"Trish M."},{"family":"Oster","given":"Robert A."},{"family":"Schuster","given":"Mindy G."},{"family":"Walker","given":"Randall"},{"family":"Walsh","given":"Thomas J."},{"family":"Wannemuehler","given":"Kathleen A."},{"family":"Chiller","given":"Tom M."}],"issued":{"date-parts":[["2010",4,15]]}},{"id":2928,"uris":["http://zotero.org/users/local/opHgB1Dy/items/G587CL67"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/G587CL67"],"itemData":{"id":2928,"type":"article-journal","abstract":"Summary\nObjectives\nThe study investigated the epidemiology and outcome of invasive aspergillosis (IA), an important cause of morbidity and mortality in immunocompromised patients.\nMethods\nCases of proven/probable IA from the Prospective Antifungal Therapy Alliance (PATH Alliance®) registry – a prospective surveillance network comprising 25 centers in the United States and Canada that collected data on invasive fungal infections from 2004 to 2008 – were analyzed with respect to clinical outcome.\nResults\nNine hundred and sixty patients with IA were enrolled, the most frequent underlying disease being hematologic malignancy (n=464 [48.3%]). Two hundred and eighty patients (29.2%) received solid organ transplant; 268 patients (27.9%) underwent hematopoietic stem cell transplantation. Identified isolates included Aspergillus fumigatus (72.6%), Aspergillus flavus (9.9%), Aspergillus niger (8.7%) and Aspergillus terreus (4.3%). The lung was most frequently affected. Following diagnosis, 47% patients received monotherapy – voriconazole (70%), an amphotericin B formulation (13.8%), or an echinocandin (10.5%) – while 279 patients (29%) received combination therapy. Twelve-week overall survival was 64.4%.\nConclusions\nIn this series of patients with IA, the lung was the predominant focus of infection, A. fumigatus was the major species isolated, and overall survival appeared slightly improved compared with previous reports.","container-title":"Journal of Infection","DOI":"10.1016/j.jinf.2012.08.003","ISSN":"0163-4453","issue":"5","journalAbbreviation":"Journal of Infection","page":"453-464","source":"ScienceDirect","title":"Clinical epidemiology of 960 patients with invasive aspergillosis from the PATH Alliance registry","volume":"65","author":[{"family":"Steinbach","given":"William J."},{"family":"Marr","given":"Kieren A."},{"family":"Anaissie","given":"Elias J."},{"family":"Azie","given":"Nkechi"},{"family":"Quan","given":"Shun-Ping"},{"family":"Meier-Kriesche","given":"Herwig-Ulf"},{"family":"Apewokin","given":"Senu"},{"family":"Horn","given":"David L."}],"issued":{"date-parts":[["2012",11,1]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}]. Approximately 15,000 U.S. hospitalizations with invasive aspergillosis are estimated to occur annually based on medical coding data, with incidence increasing over the past decade, in part because of growing numbers of patients at risk [ADDIN

ZOTERO_ITEM CSL_CITATION {"citationID":"sjxd794E","properties":{"formattedCitation":"(Benedict et al. 2019; Vallabhaneni et al. 2017)","plainCitation":"(Benedict et al. 2019; Vallabhaneni et al. 2017)","noteIndex":0},"citationItems":[{"id":2904,"uris":["http://zotero.org/users/local/opHgB1Dy/items/QW4T88BA"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/QW4T88BA"],"itemData":{"id":2904,"type":"article-journal","abstract":"To provide insight into the burden of fungal diseases in the United States, we used several administrative data sources to estimate their total direct healthcar","container-title":"Clinical Infectious Diseases","DOI":"10.1093/cid/ciy776","ISSN":"1058-4838","issue":"11","journalAbbreviation":"Clin Infect Dis","language":"en","page":"1791-1797","source":"academic.oup.com","title":"Estimation of Direct Healthcare Costs of Fungal Diseases in the United States","volume":"68","author":[{"family":"Benedict","given":"Kaitlin"},{"family":"Jackson","given":"Brendan R."},{"family":"Chiller","given":"Tom"},{"family":"Beer","given":"Karlyn D."}],"issued":{"date-parts":[["2019",5,17]]}}},{"id":1751,"uris":["http://zotero.org/users/local/opHgB1Dy/items/4G4WU3NJ"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/4G4WU3NJ"],"itemData":{"id":1751,"type":"article-journal","abstract":"AbstractBackground.. Invasive aspergillosis (IA) and mucormycosis contribute to substantial mortality, especially among immunocompromised persons, including th","container-title":"Open Forum Infectious Diseases","DOI":"10.1093/ofid/ofw268","issue":"1","journalAbbreviation":"Open Forum Infect Dis","language":"en","source":"academic.oup.com","title":"Trends in Hospitalizations Related to Invasive Aspergillosis and Mucormycosis in the United States, 2000–2013","URL":"https://academic.oup.com/ofid/article/4/1/ofw268/2901057","volume":"4","author":[{"family":"Vallabhaneni","given":"Snigdha"},{"family":"Benedict","given":"Kaitlin"},{"family":"Derado","given":"Gordana"},{"family":"Mody","given":"Rajal K."}],"accessed":{"date-parts":[["2018",9,28]]},"issued":{"date-parts":[["2017",1,1]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}]. In high risk groups, such as solid organ transplant recipients, incidence can approach 1% [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"PcS9MI5Z","properties":{"formattedCitation":"(Pappas et al. 2010)","plainCitation":"(Pappas et al. 2010)","noteIndex":0},"citationItems":[{"id":66,"uris":["http://zotero.org/users/local/opHgB1Dy/items/L8CME5VQ"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/L8CME5VQ"],"itemData":{"id":66,"type":"article-journal","abstract":"Background. Invasive fungal infections (IFIs) are a major cause of morbidity and mortality among organ transplant recipients. Multicenter prospective surveillance data to determine disease burden and secular trends are lacking.Methods. The Transplant-Associated Infection Surveillance Network (TRANSNET) is a consortium of 23 US transplant centers, including 15 that contributed to the organ transplant recipient dataset. We prospectively identified IFIs among organ transplant recipients from March, 2001 through March, 2006 at these sites. To explore trends, we calculated the 12-month cumulative incidence among 9 sequential cohorts.Results. During the surveillance period, 1208 IFIs were identified among 1063 organ transplant recipients. The most common IFIs were invasive candidiasis (53%), invasive aspergillosis (19%), cryptococcosis (8%), non-Aspergillus molds (8%), endemic fungi (5%), and zygomycosis (2%). Median time to onset of candidiasis, aspergillosis, and cryptococcosis was 103, 184, and 575 days, respectively. Among a cohort of 16,808 patients who underwent transplantation between March 2001 and September 2005 and were followed through March 2006, a total of 729 IFIs were reported among 633 persons. One-year cumulative incidences of the first IFI were 11.6%, 8.6%, 4.7%, 4.0%, 3.4%, and 1.3% for small bowel, lung, liver, heart, pancreas, and kidney transplant recipients, respectively. One-year incidence was highest for invasive candidiasis (1.95%) and aspergillosis (0.65%). Trend analysis showed a slight increase in cumulative incidence from 2002 to 2005.Conclusions. We detected a slight increase in IFIs during the surveillance period. These data provide important insights into the timing and incidence of IFIs among

organ transplant recipients, which can help to focus effective prevention and treatment strategies.","container-title":"Clinical Infectious Diseases","DOI":"10.1086/651262","ISSN":"1058-4838","issue":"8","journalAbbreviation":"Clin Infect Dis","language":"en","page":"1101-1111","source":"academic.oup.com","title":"Invasive Fungal Infections among Organ Transplant Recipients: Results of the Transplant-Associated Infection Surveillance Network (TRANSNET)","title-short":"Invasive Fungal Infections among Organ Transplant Recipients","volume":"50","author":[{"family":"Pappas","given":"Peter G."},{"family":"Alexander","given":"Barbara D."},{"family":"Andes","given":"David R."},{"family":"Hadley","given":"Susan"},{"family":"Kauffman","given":"Carol A."},{"family":"Freifeld","given":"Alison"},{"family":"Anaissie","given":"Elias J."},{"family":"Brumble","given":"Lisa M."},{"family":"Herwaldt","given":"Loreen"},{"family":"Ito","given":"James"},{"family":"Kontoyiannis","given":"Dimitrios P."},{"family":"Lyon","given":"G. Marshall"},{"family":"Marr","given":"Kieren A."},{"family":"Morrison","given":"Vicki A."},{"family":"Park","given":"Benjamin J."},{"family":"Patterson","given":"Thomas F."},{"family":"Perl","given":"Trish M."},{"family":"Oster","given":"Robert A."},{"family":"Schuster","given":"Mindy G."},{"family":"Walker","given":"Randall"},{"family":"Walsh","given":"Thomas J."},{"family":"Wannemuehler","given":"Kathleen A."},{"family":"Chiller","given":"Tom M."}],"issued":{"date-parts":[["2010",4,15]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}]. However, medical coding likely does not encompass all diagnosed cases, and the lack of national public health surveillance limits understanding of the true burden. Furthermore, many more undiagnosed cases likely exist. For example, aspergillosis is one of the leading missed diagnoses in intensive care units [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"BGk9YgVS","properties":{"formattedCitation":"(Tejerina et al. 2018,

2019)","plainCitation":"(Tejerina et al. 2018, 2019)","noteIndex":0},"citationItems":[{"id":2896,"uris":["http://zotero.org/users/local/opHgB1Dy/items/5H7NWUP8"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/5H7NWUP8"],"itemData":{"id":2896,"type":"article-journal","abstract":"Autopsy studies show that IA is among the most commonly missed diagnoses in critically ill patients. And, because of lack of unequivocal diagnostic criteria, a timely diagnosis remains challenging. We investigate the epidemiology of and the clinical risk factors for IA in critically ill patients. We conducted a retrospective, observational study of all consecutive ICU patients with evidence of IA in the postmortem examination. During the period of the study (25 years), 893 postmortem examinations were performed in the ICU. Twenty-five patients (2.8%) were diagnosed with IA in autopsy. Only ten (40%) were classified as IA ante-mortem, based on the initiation of antifungal treatment. The most common comorbid conditions were corticosteroid treatment (n = 14, 56%), chronic obstructive pulmonary disease (COPD) (n = 11, 44%), immunosuppression (n = 6, 24%) and haematological malignancy (n = 5, 20%). Twenty-three patients (92%) had three or more risk factors for IA. Critically ill patients with pulmonary infiltrates, treated with high doses intravenous corticosteroids (even for a short period of time), particularly COPD patients who developed worsening respiratory insufficiency despite appropriate treatment were at the highest risk of IA.","container-title":"Mycoses","DOI":"10.1111/myc.12927","ISSN":"1439-0507","issue":"8","language":"en","page":"673-679","source":"Wiley Online Library","title":"Invasive aspergillosis in critically ill patients: An autopsy study","title-short":"Invasive aspergillosis in critically ill patients","volume":"62","author":[{"family":"Tejerina","given":"Eva E."},{"family":"Abril","given":"Elena"},{"family":"Padilla","given":"Rebeca"},{"family":"Ruíz","given":"Covadonga Rodríguez"},{"family":"Ballen","given":"Aida"},{"family":"Frutos - Vivar","given":"Fernando"},{"family":"Lorente","given":"José Ángel"},{"family":"Esteban","given":"Andrés"}],"issued":{"date-parts":[["2019"]]}}},{"id":2909,"uris":["http://zotero.org/users/local/opHgB1Dy/items/MATYUGYI"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/MATYUGYI"],"itemData":{"id":2909,"type":"article-journal","abstract":"We evaluate the evolution over time of discrepancies between clinical diagnoses and postmortem findings in critically ill patients and assess the factors associated with these discrepancies. We conducted a prospective study of all consecutive patients who underwent autopsy in a medical-surgical intensive care unit (ICU) between January 2008 and December 2015. Among 7655 patients admitted to our ICU, 671 (8.8%) died. Clinical autopsy was performed in 215 (32%) patients. Major missed diagnoses were noted in 38 patients (17.7%). Eighteen patients (8.4%) had class I discrepancies, and 20 patients (9.3%) had class II discrepancies. The most frequently missed diagnoses were invasive aspergillosis, intestinal ischemia, myocardial infarction, cancer, and intra-abdominal abscesses. We did not find a statistically significant correlation between any premortem factor, including age, sex, severity of illness, length of hospital stay before ICU admission, length of ICU stay before death, duration of mechanical ventilation, or admitting unit, and the level of agreement between clinical and pathological diagnosis. In the last decades, the discrepancies between clinical and autopsy diagnoses persisted despite advances in medical skills and technology. Specific clinical entities such as invasive aspergillosis, mesenteric ischemia, myocardial infarction, intra-abdominal abscesses, and neoplastic diseases remain a diagnostic challenge in critically ill patients. Clinical level of diagnostic certainty does not increase with specific premortem characteristics.","container-title":"Human Pathology","DOI":"10.1016/j.humpath.2018.02.025","ISSN":"1532-8392","journalAbbreviation":"Hum. Pathol.","language":"eng","note":"PMID: 29530753","page":"85-90","source":"PubMed","title":"Autopsy-detected diagnostic errors over time in the intensive care unit","volume":"76","author":[{"family":"Tejerina","given":"Eva E."},{"family":"Padilla","given":"Rebeca"},{"family":"Abril","given":"Elena"},{"family":"Frutos-Vivar","given":"Fernando"},{"family":"Ballen","given":"Aida"},{"family":"Rodríguez-

Barbero","given":"José María"},{"family":"Lorente","given":"José Ángel"},{"family":"Esteban","given":"Andrés"}],"issued":{"date-parts":[["2018"]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}], rivaling myocardial infarction and pulmonary embolism [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"cw2jQilp","properties":{"formattedCitation":"(Winters et al. 2012)","plainCitation":"(Winters et al. 2012)","noteIndex":0},"citationItems":[{"id":1684,"uris":["http://zotero.org/users/local/opHgB1Dy/items/X9RN958W"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/X9RN958W"],"itemData":{"id":1684,"type":"article-journal","abstract":"Context Misdiagnoses may be an underappreciated cause of preventable morbidity and mortality in the intensive care unit (ICU). Their prevalence, nature, and impact remain largely unknown.\nObjectives To determine whether potentially fatal ICU misdiagnoses would be more common than in the general inpatient population (~5%), and would involve more infections or vascular events.\nData sources Systematic review of studies identified by electronic (MEDLINE, etc.) and manual searches (references in eligible articles) without language restriction (1966 through 2011).\nStudy selection and data abstraction Observational studies examining autopsy-confirmed diagnostic errors in the adult ICU were included. Studies analysing misdiagnosis of one specific disease were excluded. Study results (autopsy rate, misdiagnosis prevalence, Goldman error class, diseases misdiagnosed) were abstracted and descriptive statistics calculated. We modelled the prevalence of Class I (potentially lethal) misdiagnoses as a non-linear function of the autopsy rate.\nResults Of 276 screened abstracts, 31 studies describing 5863 autopsies (median rate 43%) were analysed. The prevalence of misdiagnoses ranged from 5.5%–100% with 28% of autopsies reporting at least one misdiagnosis and 8% identifying a Class I diagnostic error. The projected prevalence of Class I misdiagnoses for a hypothetical autopsy rate of 100% was 6.3% (95% CI 4.0% to 7.5%). Vascular events and infections were the leading lethal misdiagnoses (41% each). The most common individual Class I misdiagnoses were PE, MI, pneumonia, and aspergillosis.\nConclusions Our data suggest that as many as 40 500 adult patients in an ICU in USA may die with an ICU misdiagnoses annually. Despite this, diagnostic errors receive relatively little attention and research funding. Future studies should seek to prospectively measure the prevalence and impact of diagnostic errors and potential strategies to reduce them.","container-title":"BMJ Qual Saf","DOI":"10.1136/bmjqs-2012-000803","ISSN":"2044-5415, 2044-5423","issue":"11","journalAbbreviation":"BMJ Qual Saf","language":"en","note":"PMID: 22822241","page":"894-902","source":"qualitysafety.bmj.com","title":"Diagnostic errors in the intensive care unit: a systematic review of autopsy studies","title-short":"Diagnostic errors in the intensive care unit","volume":"21","author":[{"family":"Winters","given":"Bradford"},{"family":"Custer","given":"Jason "},{"family":"Galvagno","given":"Samuel M."},{"family":"Colantuoni","given":"Elizabeth"},{"family":"Kapoor","given":"Shruti G."},{"family":"Lee","given":"HeeWon"},{"family":"Goode","given":"Victoria"},{"family":"Robinson","given":"Karen"},{"family":"Nakhasi","given":"Atul"},{"family":"Pronovost","given":"Peter"},{"family":"Newman-Toker","given":"David"}],"issued":{"date-parts":[["2012",11,1]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}].

Aspergillus fumigatus, the species of pathogenic fungi that causes most invasive aspergillosis [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"EfsCs6fV","properties":{"formattedCitation":"(Patterson et al. 2000)","plainCitation":"(Patterson et al. 2000)","noteIndex":0},"citationItems":[{"id":2911,"uris":["http://zotero.org/users/local/opHgB1Dy/items/DU5QNJWV"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/DU5QNJWV"],"itemData":{"id":2911,"type":"article-journal","abstract":"A review of representative cases of invasive aspergillosis was conducted to describe current treatment practices and outcomes. Eighty-nine physicians experienced with aspergillosis completed case forms on 595 patients with proven or probable invasive aspergillosis diagnosed using modifications of the Mycoses Study Group criteria. Pulmonary disease was present in 56%, with disseminated infection in 19%. The major risk factors for aspergillosis were bone marrow transplantation (32%) and hematologic malignancy (29%), but patients had a variety of underlying conditions including solid organ transplants (9%), AIDS (8%), and pulmonary diseases (9%). Overall, high antifungal failure rates occurred (36%), and complete antifungal responses were noted in only 27%. Treatment practices revealed that amphotericin B alone (187 patients) was used in most severely immunosuppressed patients while itraconazole alone (58 patients) or sequential amphotericin B followed by itraconazole (93 patients) was used in patients who were less immunosuppressed than patients receiving amphotericin B alone. Response rate for patients receiving amphotericin B alone was poor, with complete responses noted in only 25% and death due to or with aspergillosis in 65%. In contrast, patients receiving itraconazole alone or following amphotericin B had death due to or with Aspergillus in 26% and 36%, respectively. These results confirm that mortality from invasive aspergillosis in severely immunosuppressed patients remains high even with standard amphotericin B. Improved responses were seen in the less immunosuppressed patients receiving sequential amphotericin B followed by itraconazole and those receiving itraconazole alone. New approaches and new therapies are needed to improve the outcome of invasive aspergillosis in high-risk patients.","container-title":"Medicine","DOI":"10.1097/00005792-200007000-00006","ISSN":"0025-7974","issue":"4","journalAbbreviation":"Medicine (Baltimore)","language":"eng","note":"PMID: 10941354","page":"250-260","source":"PubMed","title":"Invasive aspergillosis. Disease spectrum, treatment practices, and outcomes. I3 Aspergillus Study Group","volume":"79","author":[{"family":"Patterson","given":"T. F."},{"family":"Kirkpatrick","given":"W. R."},{"family":"White","given":"M."},{"family":"Hiemenz","given":"J. W."},{"family":"Wingard","given":"J.

R."},{"family":"Dupont","given":"B."},{"family":"Rinaldi","given":"M. G."},{"family":"Stevens","given":"D. A."},{"family":"Graybill","given":"J. R."}],"issued":{"date-parts":[["2000",7]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}], is common in the environment, particularly in decaying plant material but also at low levels in ambient air [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"vCaU4fNY","properties":{"formattedCitation":"(Tekaia and Latg\\uc0\\u233{} 2005)","plainCitation":"(Tekaia and Latgé 2005)","noteIndex":0},"citationItems":[{"id":3062,"uris":["http://zotero.org/users/local/opHgB1Dy/items/CP79BAEN"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/CP79BAEN"],"itemData":{"id":3062,"type":"article-journal","abstract":"Large-scale genome comparisons have shown that no gene sets are shared exclusively by both Aspergillus fumigatus and any other human pathogen sequenced to date, such as Candida or Cryptococcus species. By contrast, and in agreement with the environmental occurrence of this fungus in decaying vegetation, the enzymatic machinery required by a fungus to colonize plant substrates has been found in the A. fumigatus genome. In addition, the proteome of this fungus contains numerous efflux pumps, including >100 major facilitators that help the fungus to resist either natural aggressive molecules present in the environment or antifungal drugs in humans. Environment sensing, counteracting reactive oxidants, and retrieving essential nutriments from the environment are general metabolic traits that are associated with the growth of the saprotrophic mold A. fumigatus in an unfriendly environment such as its human host.","collection-title":"Host--microbe interactions: fungi / edited by Howard Bussey · Host--microbe interactions: parasites / edited by Artur Scherf · Host--microbe interactions: viruses / edited by Margaret CM Smith","container-title":"Current Opinion in Microbiology","DOI":"10.1016/j.mib.2005.06.017","ISSN":"1369-5274","issue":"4","journalAbbreviation":"Current Opinion in Microbiology","language":"en","page":"385-392","source":"ScienceDirect","title":"Aspergillus fumigatus: saprophyte or pathogen?","title-short":"Aspergillus fumigatus","volume":"8","author":[{"family":"Tekaia","given":"Fredj"},{"family":"Latgé","given":"Jean-Paul"}],"issued":{"date-parts":[["2005",8,1]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}]. Unlike many other fungi, it is thermotolerant and able to grow at human body temperature, including during fever response, a key factor in its human pathogenicity, as well as at elevated temperatures found in composting organic matter [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"eBiJbNJB","properties":{"formattedCitation":"(Kwon-Chung and Sugui 2013)","plainCitation":"(Kwon-Chung and Sugui 2013)","noteIndex":0},"citationItems":[{"id":3055,"uris":["http://zotero.org/users/local/opHgB1Dy/items/YZM6LPZC"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/YZM6LPZC"],"itemData":{"id":3055,"type":"article-journal","container-title":"PLoS Pathogens","DOI":"10.1371/journal.ppat.1003743","ISSN":"1553-7366","issue":"12","journalAbbreviation":"PLoS Pathog","note":"PMID: 24348239\nPMCID: PMC3857757","source":"PubMed Central","title":"Aspergillus fumigatus—What Makes the Species a Ubiquitous Human Fungal Pathogen?","URL":"https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3857757/","volume":"9","author":[{"family":"Kwon-Chung","given":"Kyung J."},{"family":"Sugui","given":"Janyce A."}],"accessed":{"date-parts":[["2019",10,22]]},"issued":{"date-parts":[["2013",12,5]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}]. Although it is widely present in agricultural areas, it is not known to cause disease in plants. Mold-active triazole antifungal medications (e.g., voriconazole) are the mainstay of treatment for invasive aspergillosis, having substantially improved patient survival following their introduction in the 1990s [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"F6nfXIMt","properties":{"formattedCitation":"(Herbrecht et al. 2002; Verweij et al.

2016a)","plainCitation":"(Herbrecht et al. 2002; Verweij et al. 2016a)","noteIndex":0},"citationItems":[{"id":244,"uris":["http://zotero.org/users/local/opHgB1Dy/items/RS7AGK55"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/RS7AGK55"],"itemData":{"id":244,"type":"article-journal","abstract":"Azole resistance in Aspergillus fumigatus has emerged as a global health problem. Although the number of cases of azole-resistant aspergillosis is still limited, resistance mechanisms continue to emerge, thereby threatening the role of the azole class in the management of diseases caused by Aspergillus. The majority of cases of azole-resistant disease are due to resistant A. fumigatus originating from the environment. Patient management is difficult due to the absence of patient risk factors, delayed diagnosis, and limited treatment options, resulting in poor treatment outcome. International and collaborative efforts are required to understand how resistance develops in the environment to allow effective measures to be implemented aimed at retaining the use of azoles both for food production and human medicine.","container-title":"Clinical Infectious Diseases","DOI":"10.1093/cid/civ885","ISSN":"1058-4838","issue":"3","journalAbbreviation":"Clin Infect Dis","language":"en","page":"362-368","source":"academic.oup.com","title":"Azole Resistance in Aspergillus fumigatus: Can We Retain the Clinical Use of Mold-Active Antifungal Azoles?","title-short":"Azole Resistance in Aspergillus fumigatus","volume":"62","author":[{"family":"Verweij","given":"Paul E."},{"family":"Chowdhary","given":"Anuradha"},{"family":"Melchers","given":"Willem J. G."},{"family":"Meis","given":"Jacques F."}],"issued":{"date-parts":[["2016",2,1]]}}},{"id":2913,"uris":["http://zotero.org/users/local/opHgB1Dy/items/TWVXWX2H"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/TWVXWX2H"],"itemData":{"id":2913,"type":"article-journal","abstract":"BACKGROUND: Voriconazole is a broad-spectrum triazole that is active against aspergillus species. We conducted a randomized trial to compare voriconazole with amphotericin B for primary therapy of invasive aspergillosis.\nMETHODS: In this randomized, unblinded trial, patients received either intravenous voriconazole (two doses of 6 mg per kilogram of body weight on day 1, then 4 mg per kilogram twice daily for at least seven days) followed by 200 mg orally twice daily or intravenous amphotericin B deoxycholate (1 to 1.5 mg per kilogram per day). Other licensed antifungal treatments were allowed if the initial therapy failed or if the patient had an intolerance to the first drug used. A complete or partial response was considered to be a successful outcome.\nRESULTS: A total of 144 patients in the voriconazole group and 133 patients in the amphotericin B group with definite or probable aspergillosis received at least one dose of treatment. In most of the patients, the underlying condition was allogeneic hematopoietic-cell transplantation, acute leukemia, or other hematologic diseases. At week 12, there were successful outcomes in 52.8 percent of the patients in the voriconazole group (complete responses in 20.8 percent and partial responses in 31.9 percent) and 31.6 percent of those in the amphotericin B group (complete responses in 16.5 percent and partial responses in 15.0 percent; absolute difference, 21.2 percentage points; 95 percent confidence interval, 10.4 to 32.9). The survival rate at 12 weeks was 70.8 percent in the voriconazole group and 57.9 percent in the amphotericin B group (hazard ratio, 0.59; 95 percent confidence interval, 0.40 to 0.88). Voriconazole-treated patients had significantly fewer severe drug-related adverse events, but transient visual disturbances were common with voriconazole (occurring in 44.8 percent of patients).\nCONCLUSIONS: In patients with invasive aspergillosis, initial therapy with voriconazole led to better responses and improved survival and resulted in fewer severe side effects than the standard approach of initial therapy with amphotericin B.","container-title":"The New England Journal of Medicine","DOI":"10.1056/NEJMoa020191","ISSN":"1533-4406","issue":"6","journalAbbreviation":"N. Engl. J. Med.","language":"eng","note":"PMID: 12167683","page":"408-415","source":"PubMed","title":"Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis","volume":"347","author":[{"family":"Herbrecht","given":"Raoul"},{"family":"Denning","given":"David W."},{"family":"Patterson","given":"Thomas F."},{"family":"Bennett","given":"John

E."},{"family":"Greene","given":"Reginald E."},{"family":"Oestmann","given":"Jörg-W."},{"family":"Kern","given":"Winfried V."},{"family":"Marr","given":"Kieren A."},{"family":"Ribaud","given":"Patricia"},{"family":"Lortholary","given":"Olivier"},{"family":"Sylvester","given":"Richard"},{"family":"Rubin","given":"Robert H."},{"family":"Wingard","given":"John R."},{"family":"Stark","given":"Paul"},{"family":"Durand","given":"Christine"},{"family":"Caillot","given":"Denis"},{"family":"Thiel","given":"Eckhard"},{"family":"Chandrasekar","given":"Pranatharthi H."},{"family":"Hodges","given":"Michael R."},{"family":"Schlamm","given":"Haran T."},{"family":"Troke","given":"Peter F."},{"family":"Pauw","given":"Ben","non-dropping-particle":"de"},{"literal":"Invasive Fungal Infections Group of the European Organisation for Research and Treatment of Cancer and the Global Aspergillus Study Group"}],"issued":{"date-parts":[["2002",8,8]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}].

Whereas relatively few fungi cause invasive disease in humans, fungi are the most common cause of plant infections. Fungicides have been widely used for centuries to treat plant infections, prevent crop loss, preserve wood and materials, and increase agricultural yield [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"4do0SvHS","properties":{"formattedCitation":"(Morton and Staub 2008; Russell 2005; Kleinkauf and European Centre for Disease Prevention and Control 2013; Wise et al. 2019; Wise and Mueller 2011)","plainCitation":"(Morton and Staub 2008; Russell 2005; Kleinkauf and European Centre for Disease Prevention and Control 2013; Wise et al. 2019; Wise and Mueller 2011)","noteIndex":0},"citationItems":[{"id":2549,"uris":["http://zotero.org/users/local/opHgB1Dy/items/LEZ4NYNL"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/LEZ4NYNL"],"itemData":{"id":2549,"type":"report","abstract":"APSnet Feature. March, 2008...Vince MortonVIVA Inc, Greensboro, NC 27410 Theodor StaubRiehen, Switzerland (both formerly Novartis Crop Protection, now Syngenta)

Corresponding author: MortV@aol.comMorton, V. and Staub, T. 2008 A Short History of Fungicides. Online, APSnet Features. doi: 10.1094/APSnetFeature-2008-0308.A c...","language":"en-US","number":"10.1094/APSnetFeature-2008-0308","publisher":"Online, APSnet Features","title":"A Short History of Fungicides","URL":"https://www.apsnet.org/edcenter/apsnetfeatures/Pages/Fungicides.aspx","author":[{"family":"Morton","given":"Vince"},{"family":"Staub","given":"Theodor"}],"accessed":{"date-parts":[["2019",7,20]]},"issued":{"date-parts":[["2008"]]}},{"id":2770,"uris":["http://zotero.org/users/local/opHgB1Dy/items/WXU9L5QM"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/WXU9L5QM"],"itemData":{"id":2770,"type":"article-journal","abstract":"Up until the 1940s chemical disease control relied upon inorganic chemical preparations, frequently prepared by the user. Key areas of use were horticulture and vegetable production with key targets being diseases that caused easily recognized damage. After this era and as the damaging effects of more crop diseases became obvious by the use of chemical control, the crop protection industry expanded rapidly and research to discover new active materials began in earnest. As new areas of chemistry were introduced, each one aiming to offer advantages over the previous ones, chemical families were born with research-based companies frequently adopting patent-busting strategies in order to capitalize on the developing fungicides market. Systemic fungicides offered new opportunities in disease control. The rise in Research and Development (R & D) and the increase in the number and quantity of chemicals being applied led to the introduction of regulation in the 1950s, initially on a voluntary basis, but now strictly controlled by legal obligations. In the 1960s, the market switched from horticulture and vegetables to one in which the main agricultural crops dominated. The cereal market, initially based on barley, moved to the current dominant market of wheat. The costs of R & D have risen dramatically in recent years and have become dominated not by the discovery process per se but by the provision of all the extra data needed to obtain registration. These rising costs happened at a time when markets showed little growth and are currently showing some decline. This has resulted in an industry that is continually striving to cut costs, normally by mergers and take-overs. As a consequence, many plant disease problems are not now being targeted by the industry and special measures have been introduced to ensure adequate disease control is available for these minor markets. Plant disease control will remain a necessity and fungicides will remain as a key factor in such control, although it is predicted that integrated control using chemicals, biological controls and biotechnology approaches will begin to dominate.","container-title":"The Journal of Agricultural Science","DOI":"10.1017/S0021859605004971","ISSN":"1469-5146, 0021-8596","issue":"1","language":"en","page":"11-25","source":"Cambridge Core","title":"A century of fungicide evolution","volume":"143","author":[{"family":"Russell","given":"P. E."}],"issued":{"date-parts":[["2005",2]]}}},{"id":1086,"uris":["http://zotero.org/users/local/opHgB1Dy/items/3628VSAE"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/3628VSAE"],"itemData":{"id":1086,"type":"book","collection-title":"Technical report / European Centre for Disease Prevention and Control","event-place":"Stockholm","ISBN":"978-92-9193-444-7","language":"en","note":"OCLC: 855553214","number-of-pages":"17","publisher":"ECDC [u.a.] Europäisches Zentrum für die Prävention und die Kontrolle von Krankheiten","publisher-place":"Stockholm","source":"Gemeinsamer Bibliotheksverbund ISBN","title":"Risk assessment on the impact of environmental usage of triazoles on the development and spread of resistance to medical triazoles in Aspergillus species","editor":[{"family":"Kleinkauf","given":"Niels"},{"literal":"European Centre for Disease Prevention and Control"}],"issued":{"date-parts":[["2013"]]}}},{"id":2841,"uris":["http://zotero.org/users/local/opHgB1Dy/items/MB62XFGP"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/MB62XFGP"],"itemData":{"id":2841,"type":"article-journal","container-title":"APSnet Feature Articles","DOI":"10.1094/APSnetFeature-2011-0531","ISSN":"2153-0297","journalAbbreviation":"APSnet Features","language":"en","source":"DOI.org

(Crossref)","title":"Are Fungicides No Longer Just For Fungi? An Analysis of Foliar Fungicide Use in Corn","title-short":"Are Fungicides No Longer Just For Fungi?","URL":"http://www.apsnet.org/publications/apsnetfeatures/Pages/fungicide.aspx","author":[{"family":"Wise","given":"Kiersten"},{"family":"Mueller","given":"Daren"}],"accessed":{"date-parts":[["2019",9,1]]},"issued":{"date-parts":[["2011"]]}}},{"id":2636,"uris":["http://zotero.org/users/local/opHgB1Dy/items/NQWIATN2"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/NQWIATN2"],"itemData":{"id":2636,"type":"article-journal","abstract":"Background Foliar fungicide applications to corn (Zea mays L.) occur at one or more application timings ranging from early vegetative growth stages to mid-reproductive stages. Previous studies indicated that fungicide applications are profitable under high disease pressure when applied during the tasseling to silking growth stages. Few comprehensive studies in corn have examined the impact of fungicide applications at an early vegetative growth stage (V6) compared to late application timings (VT) for yield response and return on fungicide investment (ROI) across multiple locations.","container-title":"PLOS ONE","DOI":"10.1371/journal.pone.0217510","ISSN":"1932-6203","issue":"6","journalAbbreviation":"PLoS ONE","language":"en","page":"e0217510","source":"DOI.org (Crossref)","title":"Meta-analysis of yield response of foliar fungicide-treated hybrid corn in the United States and Ontario, Canada","volume":"14","author":[{"family":"Wise","given":"Kiersten A."},{"family":"Smith","given":"Damon"},{"family":"Freije","given":"Anna"},{"family":"Mueller","given":"Daren S."},{"family":"Kandel","given":"Yuba"},{"family":"Allen","given":"Tom"},{"family":"Bradley","given":"Carl A."},{"family":"Byamukama","given":"Emmanuel"},{"family":"Chilvers","given":"Martin"},{"family":"Faske","given":"Travis"},{"family":"Friskop","given":"Andrew"},{"family":"Hollier","given":"Clayton"},{"family

":"Jackson-Ziems","given":"Tamra A."},{"family":"Kelly","given":"Heather"},{"family":"Kemerait","given":"Bob"},{"family":"Price","given":"Paul"},{"family":"Robertson","given":"Alison"},{"family":"Tenuta","given":"Albert"}],"editor":[{"family":"Lightfoot","given":"David A."}],"issued":{"date-parts":[["2019",6,5]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}]. Triazoles are one of the most widely used agricultural fungicide classes, comprising over a quarter of estimated global fungicide sales [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"6etgml0P","properties":{"formattedCitation":"(Kleinkauf and European Centre for Disease Prevention and Control 2013)","plainCitation":"(Kleinkauf and European Centre for Disease Prevention and Control 2013)","noteIndex":0},"citationItems":[{"id":1086,"uris":["http://zotero.org/users/local/opHgB1Dy/items/3628VSAE"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/3628VSAE"],"itemData":{"id":1086,"type":"book","collection-title":"Technical report / European Centre for Disease Prevention and Control","event-place":"Stockholm","ISBN":"978-92-9193-444-7","language":"en","note":"OCLC: 855553214","number-of-pages":"17","publisher":"ECDC [u.a.] Europäisches Zentrum für die Prävention und die Kontrolle von Krankheiten","publisher-place":"Stockholm","source":"Gemeinsamer Bibliotheksverbund ISBN","title":"Risk assessment on the impact of environmental usage of triazoles on the development and spread of resistance to medical triazoles in Aspergillus species","editor":[{"family":"Kleinkauf","given":"Niels"},{"literal":"European Centre for Disease Prevention and Control"}],"issued":{"date-parts":[["2013"]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}]. Fungal pathogens of plants have developed resistance to agricultural fungicides, including triazoles [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"9is8mkki","properties":{"formattedCitation":"(Cools and Fraaije 2008; Hu et al. 2016; Price et al. 2015)","plainCitation":"(Cools and Fraaije 2008; Hu et al. 2016; Price et al.

2015)","noteIndex":0},"citationItems":[{"id":3158,"uris":["http://zotero.org/users/local/opHgB1Dy/items/MN8UE7JN"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/MN8UE7JN"],"itemData":{"id":3158,"type":"article-journal","abstract":"There has been a recent rapid decline in the efficacy of some, but not all, azole fungicides in controlling the Septoria leaf blotch pathogen of wheat, Mycosphaerella graminicola. Hans J. Cools and Bart A. Fraaije ask the question: can widespread resistance to all azoles develop in this pathogen? Copyright © 2008 Society of Chemical Industry","container-title":"Pest Management Science","DOI":"10.1002/ps.1568","ISSN":"1526-4998","issue":"7","language":"en","page":"681-684","source":"Wiley Online Library","title":"Are azole fungicides losing ground against Septoria wheat disease? Resistance mechanisms in Mycosphaerella graminicola","title-short":"Are azole fungicides losing ground against Septoria wheat disease?","volume":"64","author":[{"family":"Cools","given":"Hans J."},{"family":"Fraaije","given":"Bart A."}],"issued":{"date-parts":[["2008"]]}},{"id":3319,"uris":["http://zotero.org/users/local/opHgB1Dy/items/X6RHBGY3"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/X6RHBGY3"],"itemData":{"id":3319,"type":"article-journal","abstract":"Previous research has shown that Botrytis cinerea isolates with resistance to multiple chemical classes of fungicides exist in eastern strawberry fields. In this study, the fungicide resistance profiles of 2,130 isolates from flowers of commercial strawberry fields located in multiple states was determined over four consecutive strawberry production seasons. Producers were asked to alternate single-site fungicides that were considered low risk in their specific location based on resistance monitoring results in their fields. This recommendation led to an increase of chemical class diversity used in the spray programs. Results indicated that simultaneous resistance in individual isolates to two, three, four, five, six, and seven classes of fungicides increased over time. The increase in chemical class resistances within isolates was likely due to a process we termed "selection by association", where fungicide resistance traits were often linked to the trait being selected rather than the selectable trait itself. Data analysis also indicated that the odds were highest for isolates resistant to one chemical class (1CCR) to be resistant to thiophanate-methyl; for 2CCR isolates to be resistant to thiophanate-methyl and pyraclostrobin; and for 3CCR isolates to be resistant to thiophanate-methyl, pyraclostrobin, and either cyprodinil or fenhexamid. We hypothesize that the more chemical classes are used in a spray program, the faster isolates will be selected with increasing numbers of chemical class resistances by virtue of selection by association if such isolates preexist in the population.","container-title":"Phytopathology™","DOI":"10.1094/PHYTO-04-16-0161-R","ISSN":"0031-949X","issue":"12","journalAbbreviation":"Phytopathology™","page":"1513-1520","source":"apsjournals.apsnet.org (Atypon)","title":"Resistance to Increasing Chemical Classes of Fungicides by Virtue of “Selection by Association” in Botrytis cinerea","volume":"106","author":[{"family":"Hu","given":"Meng-Jun"},{"family":"Cox","given":"Kerik D."},{"family":"Schnabel","given":"Guido"}],"issued":{"date-parts":[["2016",8,9]]}}},{"id":2280,"uris":["http://zotero.org/users/local/opHgB1Dy/items/D4RSCXW3"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/D4RSCXW3"],"itemData":{"id":2280,"type":"article-journal","abstract":"Plant fungal pathogens can have devastating effects on a wide range of crops, including cereals and fruit (such as wheat and grapes), causing losses in crop yield, which are costly to the agricultural economy and threaten food security. Azole antifungals are the treatment of choice; however, resistance has arisen against these compounds, which could lead to devastating consequences. Therefore, it is important to understand how these fungicides are used and how the resistance arises in order to tackle the problem fully. Here, we give an overview of the problem and discuss the mechanisms that mediate azole resistance in agriculture (point mutations in the CYP51 amino acid sequence, overexpression of the CYP51 enzyme and overexpression of genes encoding efflux pump proteins). © 2015 Society of Chemical Industry.","container-title":"Pest management science","DOI":"10.1002/ps.4029","issue":"8","page":"1054-1058","source":"Semantic

Scholar","title":"Azole fungicides - understanding resistance mechanisms in agricultural fungal pathogens.","volume":"71","author":[{"family":"Price","given":"Claire Louise"},{"family":"Parker","given":"Josie Elizabeth"},{"family":"Warrilow","given":"Andrew Graham Samuel"},{"family":"Kelly","given":"Diane"},{"family":"Kelly","given":"Steven L."}],"issued":{"date-parts":[["2015"]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}], prompting the Fungicide Resistance Action Committee (FRAC) and other organizations to devote substantial resources to preventing and managing resistance [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"uBpcLKsH","properties":{"formattedCitation":"(FRAC | Home)","plainCitation":"(FRAC | Home)","noteIndex":0},"citationItems":[{"id":3048,"uris":["http://zotero.org/users/local/opHgB1Dy/items/WGSEWW6C"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/WGSEWW6C"],"itemData":{"id":3048,"type":"webpage","title":"FRAC | Home","URL":"https://www.frac.info/home","accessed":{"date-parts":[["2019",10,21]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}]. Notably, certain agricultural triazole fungicides, including difenoconazole, propiconazole, tebuconazole, bromuconazole, and epoxiconazole, are structurally highly similar to medical triazoles used to treat aspergillosis (e.g., voriconazole, itraconazole, and posaconazole) [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"k9cPcMsc","properties":{"formattedCitation":"(Snelders et al. 2012)","plainCitation":"(Snelders et al. 2012)","noteIndex":0},"citationItems":[{"id":2272,"uris":["http://zotero.org/users/local/opHgB1Dy/items/XSZI44QZ"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/XSZI44QZ"],"itemData":{"id":2272,"type":"article-journal","abstract":"Background Azoles play an important role in the management of Aspergillus diseases. Azole resistance is an emerging global problem in Aspergillus fumigatus, and may develop through patient therapy. In addition, an environmental route of resistance development has been suggested through exposure to 14α-demethylase inhibitors (DMIs). The main resistance mechanism associated with this putative fungicide-driven route is a combination of alterations in the Cyp51A-gene (TR34/L98H). We investigated if TR34/L98H could have developed through exposure to DMIs. Methods and Findings Thirty-one compounds that have been authorized for use as fungicides, herbicides, herbicide safeners and plant growth regulators in the Netherlands between 1970 and 2005, were investigated for cross-resistance to medical triazoles. Furthermore, CYP51-protein homology modeling and molecule alignment studies were performed to identify similarity in molecule structure and docking modes. Five triazole DMIs, propiconazole, bromuconazole, tebuconazole, epoxiconazole and difenoconazole, showed very similar molecule structures to the medical triazoles and adopted similar poses while docking the protein. These DMIs also showed the greatest cross-resistance and, importantly, were authorized for use between 1990 and 1996, directly preceding the recovery of the first clinical TR34/L98H isolate in 1998. Through microsatellite genotyping of TR34/L98H isolates we were able to calculate that the first isolate would have arisen in 1997, confirming the results of the abovementioned experiments. Finally, we performed induction experiments to investigate if TR34/L98H could be induced under laboratory conditions. One isolate evolved from two copies of the tandem repeat to three, indicating that fungicide pressure can indeed result in these genomic changes. Conclusions Our findings support a fungicide-driven route of TR34/L98H development in A. fumigatus. Similar molecule structure characteristics of five triazole DMIs and the three medical triazoles appear the underlying mechanism of cross resistance development. Our findings have major implications for the assessment of health risks associated with the use of triazole DMIs.","container-title":"PLOS ONE","DOI":"10.1371/journal.pone.0031801","ISSN":"1932-6203","issue":"3","journalAbbreviation":"PLOS ONE","language":"en","page":"e31801","source":"PLoS Journals","title":"Triazole Fungicides Can Induce Cross-Resistance to Medical Triazoles in Aspergillus

fumigatus","volume":"7","author":[{"family":"Snelders","given":"Eveline"},{"family":"Camps","given":"Simone M.
T."},{"family":"Karawajczyk","given":"Anna"},{"family":"Schaftenaar","given":"Gijs"},{"family":"Kema","given":"Gert H. J."},{"family":"Lee","given":"Henrich A.","dropping-particle":"van der"},{"family":"Klaassen","given":"Corné H."},{"family":"Melchers","given":"Willem J.
G."},{"family":"Verweij","given":"Paul E."}],"issued":{"date-parts":[["2012",3,1]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}].

Like plant pathogens that have developed resistance to triazole fungicides, *A. fumigatus* strains resistant to medical triazoles have emerged globally, prompting public health concerns. Resistant aspergillosis is associated with treatment failure and high mortality, up to 88% [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"neZBlX05","properties":{"formattedCitation":"(Lestrade et al. 2019; van der Linden et al. 2011)","plainCitation":"(Lestrade et al. 2019; van der Linden et al. 2011)","noteIndex":0},"citationItems":[{"id":2708,"uris":["http://zotero.org/users/local/opHgB1Dy/items/F8N7JQVC"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/F8N7JQVC"],"itemData":{"id":2708,"type":"article-journal","abstract":"A multicenter, retrospective, cohort study showed a 21% higher day 42 mortality in voriconazole-resistant invasive aspergillosis compared with voriconazole-susc","container-title":"Clinical Infectious Diseases","DOI":"10.1093/cid/ciy859","ISSN":"1058-4838","issue":"9","journalAbbreviation":"Clin Infect Dis","language":"en","page":"1463-1471","source":"academic.oup.com","title":"Voriconazole Resistance and Mortality in Invasive Aspergillosis: A Multicenter Retrospective Cohort Study","title-short":"Voriconazole Resistance and Mortality in Invasive Aspergillosis","volume":"68","author":[{"family":"Lestrade","given":"Pieter P."},{"family":"Bentvelsen","given":"Robbert G."},{"family":"Schauwvlieghe","given":"Alexander F. A.

D."},{"family":"Schalekamp","given":"Steven"},{"family":"Velden","given":"Walter J. F. M.","non-dropping-particle":"van der"},{"family":"Kuiper","given":"Ed J."},{"family":"Paassen","given":"Judith","non-dropping-particle":"van"},{"family":"Hoven","given":"Ben","non-dropping-particle":"van der"},{"family":"Lee","given":"Henrich A.","non-dropping-particle":"van der"},{"family":"Melchers","given":"Willem J. G."},{"family":"Haan","given":"Anton F.","non-dropping-particle":"de"},{"family":"Hoeven","given":"Hans L.","non-dropping-particle":"van der"},{"family":"Rijnders","given":"Bart J. A."},{"family":"Beek","given":"Martha T.","non-dropping-particle":"van der"},{"family":"Verweij","given":"Paul E."}],"issued":{"date-parts":[["2019",4,24]]}}},{"id":2257,"uris":["http://zotero.org/users/local/opHgB1Dy/items/4E2AR8VY"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/4E2AR8VY"],"itemData":{"id":2257,"type":"article-journal","abstract":"The prevalence and spread of azole resistance in clinical Aspergillus fumigatus isolates in the Netherlands are currently unknown. Therefore, we perfo...","DOI":"10.3201/eid1710.110226","language":"en-us","source":"wwwnc.cdc.gov","title":"Clinical Implications of Azole Resistance in Aspergillus fumigatus, the Netherlands, 2007–2009 - Volume 17, Number 10—October 2011 - Emerging Infectious Diseases journal - CDC","URL":"https://wwwnc.cdc.gov/eid/article/17/10/11-0226_article","author":[{"family":"Linden","given":"Jan W. M.","non-dropping-particle":"van der"},{"family":"Snelders","given":"Eveline"},{"family":"Kampinga","given":"Greetje A."},{"family":"Rijnders","given":"Bart J. A."},{"family":"Mattsson","given":"Eva"},{"family":"Debets-Ossenkopp","given":"Yvette J."},{"family":"Kuijper","given":"Ed J."},{"family":"Tiel","given":"Frank H. Van"},{"family":"Melchers","given":"Willem J. G."},{"family":"Verweij","given":"Paul E."}],"accessed":{"date-parts":[["2019",7,20]]},"issued":{"date-parts":[["2011"]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}]. Death occurs more commonly in resistant infections, with 90-day mortality being 25% higher in patients with resistant vs. susceptible aspergillosis in a European study [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"xQF55GYR","properties":{"formattedCitation":"(Lestrade et al. 2019)","plainCitation":"(Lestrade et al. 2019)","noteIndex":0},"citationItems":[{"id":2708,"uris":["http://zotero.org/users/local/opHgB1Dy/items/F8N7JQVC"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/F8N7JQVC"],"itemData":{"id":2708,"type":"article-journal","abstract":"A multicenter, retrospective, cohort study showed a 21% higher day 42 mortality in voriconazole-resistant invasive aspergillosis compared with voriconazole-susc","container-title":"Clinical Infectious Diseases","DOI":"10.1093/cid/ciy859","ISSN":"1058-4838","issue":"9","journalAbbreviation":"Clin Infect Dis","language":"en","page":"1463-1471","source":"academic.oup.com","title":"Voriconazole Resistance and Mortality in Invasive Aspergillosis: A Multicenter Retrospective Cohort Study","title-short":"Voriconazole Resistance and Mortality in Invasive Aspergillosis","volume":"68","author":[{"family":"Lestrade","given":"Pieter P."},{"family":"Bentvelsen","given":"Robbert G."},{"family":"Schauwvlieghe","given":"Alexander F. A. D."},{"family":"Schalekamp","given":"Steven"},{"family":"Velden","given":"Walter J. F. M.","non-dropping-particle":"van der"},{"family":"Kuiper","given":"Ed J."},{"family":"Paassen","given":"Judith","non-dropping-particle":"van"},{"family":"Hoven","given":"Ben","non-dropping-particle":"van der"},{"family":"Lee","given":"Henrich A.","non-dropping-particle":"van der"},{"family":"Melchers","given":"Willem J. G."},{"family":"Haan","given":"Anton F.","non-dropping-particle":"de"},{"family":"Hoeven","given":"Hans L.","non-dropping-particle":"van der"},{"family":"Rijnders","given":"Bart J. A."},{"family":"Beek","given":"Martha T.","non-dropping-particle":"van der"},{"family":"Verweij","given":"Paul E."}],"issued":{"date-parts":[["2019",4,24]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}]. Resistance in *A. fumigatus* can develop in two ways. First, it can develop inside the body under selection pressure from long-term use of triazole medications. During the 1990s, small numbers of triazole-resistant infections were identified in patients who were receiving long-term triazole prophylaxis or therapy (e.g., for aspergilloma, cavitary lung disease, or other non-invasive aspergillosis), with resistance mechanisms involving point mutations in the triazole target and ergosterol synthesis gene, *CYP51A* [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"cu9nQ9qF","properties":{"formattedCitation":"(Camps et al. 2012; Heo et al. 2017; Howard et al. 2013, 2009)","plainCitation":"(Camps et al. 2012; Heo et al. 2017; Howard et al. 2013, 2009)","noteIndex":0},"citationItems":[{"id":2759,"uris":["http://zotero.org/users/local/opHgB1Dy/items/4QDWKHKH"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/4QDWKHKH"],"itemData":{"id":2759,"type":"article-journal","abstract":"An increase in the frequency of azole-resistant Aspergillus fumigatus has emerged., Azoles are the mainstay of oral therapy for aspergillosis. Azole resistance in Aspergillus has been reported infrequently. The first resistant isolate was detected in 1999 in Manchester, UK. In a clinical collection of 519 A. fumigatus isolates, the frequency of itraconazole resistance was 5%, a significant increase since 2004 (p<0.001). Of the 34 itraconazole-resistant isolates we studied, 65% (22) were cross-resistant to voriconazole and 74% (25) were cross-resistant to posaconazole. Thirteen of 14 evaluable patients in our study had prior azole exposure; 8 infections failed therapy (progressed), and 5 failed to improve (remained stable). Eighteen amino acid alterations were found in the target enzyme, Cyp51A, 4 of which were novel. A population genetic analysis of microsatellites showed the existence of resistant mutants that evolved from originally susceptible strains, different cyp51A mutations in the same strain, and microalterations in microsatellite repeat number. Azole resistance in A. fumigatus is an emerging problem and may develop during azole therapy.","container-title":"Emerging Infectious Diseases","DOI":"10.3201/eid1507.090043","ISSN":"1080-

6040","issue":"7","journalAbbreviation":"Emerg Infect Dis","note":"PMID: 19624922\nPMCID: PMC2744247","page":"1068-1076","source":"PubMed Central","title":"Frequency and Evolution of Azole Resistance in Aspergillus fumigatus Associated with Treatment Failure1","volume":"15","author":[{"family":"Howard","given":"Susan J."},{"family":"Cerar","given":"Dasa"},{"family":"Anderson","given":"Michael J."},{"family":"Albarrag","given":"Ahmed"},{"family":"Fisher","given":"Matthew C."},{"family":"Pasqualotto","given":"Alessandro C."},{"family":"Laverdiere","given":"Michel"},{"family":"Arendrup","given":"Maiken C."},{"family":"Perlin","given":"David S."},{"family":"Denning","given":"David W."}],"issued":{"date-parts":[["2009",7]]}}},{"id":2815,"uris":["http://zotero.org/users/local/opHgB1Dy/items/Z3UE8BLP"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/Z3UE8BLP"],"itemData":{"id":2815,"type":"article-journal","abstract":"Nine consecutive isogenic Aspergillus fumigatus isolates cultured from a patient with aspergilloma were investigated for azole resistance. The first cultured isolate showed a wild-type phenotype, but four azole-resistant phenotypes were observed in the subsequent eight isolates. Four mutations were found in the cyp51A gene of these isolates, leading to the substitutions A9T, G54E, P216L, and F219I. Only G54 substitutions were previously proved to be associated with azole resistance. Using a Cyp51A homology model and recombination experiments in which the mutations were introduced into a susceptible isolate, we show that the substitutions at codons P216 and F219 were both associated with resistance to itraconazole and posaconazole. A9T was also present in the wild-type isolate and thus considered a Cyp51A polymorphism. Isolates harboring F219I evolved further into a pan-azole-resistant phenotype, indicating an additional acquisition of a non-Cyp51A-mediated resistance mechanism. Review of the literature showed that in patients who develop azole resistance during therapy, multiple resistance mechanisms commonly emerge. Furthermore, the median time between the last cultured wild-type isolate and the first azole-resistant isolate was 4 months (range, 3 weeks to 23 months), indicating a rapid induction of resistance.","container-title":"Antimicrobial Agents and Chemotherapy","DOI":"10.1128/AAC.05088-11","ISSN":"0066-4804, 1098-6596","issue":"1","language":"en","note":"PMID: 22005994","page":"10-16","source":"aac.asm.org","title":"Rapid Induction of Multiple Resistance Mechanisms in Aspergillus fumigatus during Azole Therapy: a Case Study and Review of the Literature","title-short":"Rapid Induction of Multiple Resistance Mechanisms in Aspergillus fumigatus during Azole Therapy","volume":"56","author":[{"family":"Camps","given":"Simone M. T."},{"family":"Linden","given":"Jan W. M.","dropping-particle":"van der"},{"family":"Li","given":"Yi"},{"family":"Kuijper","given":"Ed J."},{"family":"Dissel","given":"Jaap T.","dropping-particle":"van"},{"family":"Verweij","given":"Paul E."},{"family":"Melchers","given":"Willem J. G."}],"issued":{"date-parts":[["2012",1,1]]}}},{"id":2821,"uris":["http://zotero.org/users/local/opHgB1Dy/items/ABVI7ZEX"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/ABVI7ZEX"],"itemData":{"id":2821,"type":"article-journal","abstract":"Aspergillomas develop from progressive layers of mycelial growth on the walls of pulmonary cavities over months. Aspergillomas are characteristic of chronic pulmonary aspergillosis and are a risk factor for azole resistance. We investigated genotypic and phenotypic alterations in Aspergillus fumigatus recovered from aspergillomas. Aspergillomas were removed from three patients (two at surgery, one at autopsy) and dissected. Overall 92 colonies of A. fumigatus were isolated. Microsatellite typing was conducted to determine genetic type. Itraconazole, voriconazole and posaconazole susceptibilities were performed. The cyp51A gene was sequenced in 22 isolates. Isolates from Patient 1 (n = 25) were azole susceptible and resistant, although all cyp51A sequences were wild type, the isolates split into two distinct clades. In Patient 2, isolates were less variable (n = 10), all were azole susceptible. In Patient 3 only azole-resistant strains (n = 57) were isolated, with M220K or M220T Cyp51A alterations, and microevolution was indicated. Marked diversity was observed in isolates from these patients; revealing differences in azole susceptibility, mechanism of resistance and genetic type. Importantly, routine sampling from respiratory specimens proved suboptimal in all cases; azole resistance was missed (Patient 1), cultures were negative (Patient 2) and high-level posaconazole resistance was not detected (Patient 3).","container-title":"Mycoses","DOI":"10.1111/myc.12047","ISSN":"1439-0507","issue":"4","journalAbbreviation":"Mycoses","language":"eng","note":"PMID: 23369025","page":"434-441","source":"PubMed","title":"Major variations in Aspergillus fumigatus arising within aspergillomas in chronic pulmonary aspergillosis","volume":"56","author":[{"family":"Howard","given":"S. J."},{"family":"Pasqualotto","given":"A. C."},{"family":"Anderson","given":"M. J."},{"family":"Leatherbarrow","given":"H."},{"family":"Albarrag","given":"A. M."},{"family":"Harrison","given":"E."},{"family":"Gregson","given":"L."},{"family":"Bowyer","given":"P."},{"family":"Denning","given":"D. W."}],"issued":{"date-parts":[["2013",7]]}}},{"id":2919,"uris":["http://zotero.org/users/local/opHgB1Dy/items/6CAU6JR4"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/6CAU6JR4"],"itemData":{"id":2919,"type":"article-journal","abstract":"Background: Azole-resistant aspergillosis in high-risk patients with hematological malignancy or hematopoietic stem cell transplantation (HSCT) is a cause of concern.\nMethods: We examined changes over time in triazole minimum inhibitory concentrations (MICs) of 290 sequential Aspergillus isolates recovered from respiratory sources during 1999-2002 (before introduction of the Aspergillus-potent triazoles voriconazole and posaconazole) and 2003-2015 at MD Anderson Cancer Center. We also tested for polymorphisms in ergosterol biosynthetic genes (cyp51A, erg3C, erg1) in the 37 Aspergillus fumigatus isolates isolated from both periods that had non-wild-type (WT) MICs. For the 107 patients with hematologic cancer and/or HSCT with invasive pulmonary aspergillosis, we correlated in vitro susceptibility with 42-day mortality.\nResults: Non-WT MICs were found in 37 (13%) isolates and was only low level (MIC <8 mg/L) in all isolates. Higher-triazole MICs were more frequent in the second period and were Aspergillus-species specific, and only encountered in A. fumigatus. No polymorphisms in cyp51A, erg3C, erg1 genes were identified. There was no correlation between in vitro MICs with 42-day mortality in patients with invasive pulmonary aspergillosis, irrespective of antifungal treatment. Asian race (odds ratio [OR], 20.9; 95% confidence interval [CI], 2.5-173.5; P = .005) and azole exposure in the prior 3 months (OR, 9.6; 95% CI, 1.9-48.5; P = .006) were associated with azole resistance.\nConclusions: Non-WT azole MICs in Aspergillus are increasing and this is associated with prior azole exposure in patients with hematologic cancer or HSCT. However, no correlation of MIC with outcome of aspergillosis was found in our patient cohort.","container-title":"Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America","DOI":"10.1093/cid/cix297","ISSN":"1537-6591","issue":"2","journalAbbreviation":"Clin. Infect. Dis.","language":"eng","note":"PMID: 28379304\nPMCID: PMC5850538","page":"216-225","source":"PubMed","title":"Changes in In Vitro Susceptibility Patterns of Aspergillus to Triazoles and Correlation With Aspergillosis Outcome in a Tertiary Care Cancer Center, 1999-2015","volume":"65","author":[{"family":"Heo","given":"Sang Taek"},{"family":"Tatara","given":"Alexander M."},{"family":"Jiménez-Ortigosa","given":"Cristina"},{"family":"Jiang","given":"Ying"},{"family":"Lewis","given":"Russell E."},{"family":"Tarrand","given":"Jeffrey"},{"family":"Tverdek","given":"Frank"},{"family":"Albert","given":"Nathaniel D."},{"family":"Verweij","given":"Paul E."},{"family":"Meis","given":"Jacques F."},{"family":"Mikos","given":"Antonios G."},{"family":"Perlin","given":"David S."},{"family":"Kontoyiannis","given":"Dimitrios P."}],"issued":{"date-parts":[["2017",7,15]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}]. Resistance occurs less frequently in invasive aspergillosis, presumably because the fungus has less time to grow in the body. Second, resistance can

develop in the environment following long-term use of fungicides on plants. People are then exposed to resistant strains, typically through inhalation. In the late 1990s, a new resistance mechanism was identified in patients who had *A. fumigatus* infections, and the same mechanism was identified in *A. fumigatus* exposed to triazole fungicides in the environment. This mechanism, TR_{34}/L98H (which we will refer to as TR_{34}), includes a 34-base pair tandem repeat (TR) in the *cyp51A* promoter coupled with a specific point mutation in the coding region and can confer resistance to all triazole medications, known as pan-resistance [ADDIN ZOTERO_ITEM CSL_CITATION
{"citationID":"Tib7bk7J","properties":{"formattedCitation":"(Abdolrasouli et al. 2018)","plainCitation":"(Abdolrasouli et al. 2018)","noteIndex":0},"citationItems":[{"id":2423,"uris":["http://zotero.org/users/local/opHgB1Dy/items/W57KMETM"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/W57KMETM"],"itemData":{"id":2423,"type":"article-journal","abstract":"Background/objectives: Aspergillus fumigatus is the leading cause of invasive aspergillosis. Treatment is hindered by the emergence of resistance to triazole antimycotic agents. Here, we present the prevalence of triazole resistance among clinical isolates at a major centralized medical mycology laboratory in London, United Kingdom, in the period 1998 – 2017. Methods: A large number (n = 1469) of clinical A. fumigatus isolates from unselected clinical specimens were identified and their susceptibility against three triazoles, amphotericin B and three echinocandin agents was carried out. All isolates were identified phenotypically and antifungal susceptibility testing was carried out by using a standard broth microdilution method. Results: Retrospective surveillance (1998-2011) shows 5/1151 (0.43%) isolates were resistant to at least one of the clinically used triazole antifungal agents. Prospective surveillance (2015-2017) shows 7/356 (2.2%) isolates were resistant to at least one triazole antifungals demonstrating an increase in incidence of triazole-resistant A. fumigatus in our laboratory. Among five isolates collected from 2015 to 2017 and available for molecular testing, three harboured TR34/L98H alteration in the cyp51A gene that are associated with the acquisition of

resistance in the non-patient environment. Conclusions: These data show that historically low prevalence of azole resistance may be increasing, warranting further surveillance of susceptible patients.","container-title":"Frontiers in Microbiology","DOI":"10.3389/fmicb.2018.02234","ISSN":"1664-302X","journalAbbreviation":"Front. Microbiol.","language":"English","source":"Frontiers","title":"Surveillance for Azole-Resistant Aspergillus fumigatus in a Centralized Diagnostic Mycology Service, London, United Kingdom, 1998–2017","URL":"https://www.frontiersin.org/articles/10.3389/fmicb.2018.02234/full","volume":"9","author":[{"family":"Abdolrasouli","given":"Alireza"},{"family":"Petrou","given":"Michael A."},{"family":"Park","given":"Hyun"},{"family":"Rhodes","given":"Johanna L."},{"family":"Rawson","given":"Timothy M."},{"family":"Moore","given":"Luke S. P."},{"family":"Donaldson","given":"Hugo"},{"family":"Holmes","given":"Alison H."},{"family":"Fisher","given":"Matthew C."},{"family":"Armstrong-James","given":"Darius"}],"accessed":{"date-parts":[["2019",7,20]]},"issued":{"date-parts":[["2018"]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}]. In contrast to the resistance mechanism that can develop inside the human body, this environmental resistance was observed in isolates primarily from patients who have never taken triazole medicines [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"2TLMIrkW","properties":{"formattedCitation":"(Snelders et al. 2008; Verweij et al. 2007)","plainCitation":"(Snelders et al. 2008; Verweij et al. 2007)","noteIndex":0},"citationItems":[{"id":2828,"uris":["http://zotero.org/users/local/opHgB1Dy/items/THKVJAWQ"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/THKVJAWQ"],"itemData":{"id":2828,"type":"article-journal","abstract":"Background Resistance to triazoles was recently reported in Aspergillus fumigatus isolates cultured from patients with invasive aspergillosis. The prevalence of azole resistance in A. fumigatus is unknown. We investigated the prevalence and spread of azole resistance using our culture collection that contained A. fumigatus isolates collected between 1994 and 2007. Methods and Findings We investigated the prevalence of itraconazole (ITZ) resistance in 1,912 clinical A. fumigatus isolates collected from 1,219 patients in our University Medical Centre over a 14-y period. The spread of resistance was investigated by analyzing 147 A. fumigatus isolates from 101 patients, from 28 other medical centres in The Netherlands and 317 isolates from six other countries. The isolates were characterized using phenotypic and molecular methods. The electronic patient files were used to determine the underlying conditions of the patients and the presence of invasive aspergillosis. ITZ-resistant isolates were found in 32 of 1,219 patients. All cases were observed after 1999 with an annual prevalence of 1.7% to 6%. The ITZ-resistant isolates also showed elevated minimum inhibitory concentrations of voriconazole, ravuconazole, and posaconazole. A substitution of leucine 98 for histidine in the cyp51A gene, together with two copies of a 34-bp sequence in tandem in the gene promoter (TR/L98H), was found to be the dominant resistance mechanism. Microsatellite analysis indicated that the ITZ-resistant isolates were genetically distinct but clustered. The ITZ-sensitive isolates were not more likely to be responsible for invasive aspergillosis than the ITZ-resistant isolates. ITZ resistance was found in isolates from 13 patients (12.8%) from nine other medical centres in The Netherlands, of which 69% harboured the TR/L98H substitution, and in six isolates originating from four other countries. Conclusions Azole resistance has emerged in A. fumigatus and might be more prevalent than currently acknowledged. The presence of a dominant resistance mechanism in clinical isolates suggests that isolates with this mechanism are spreading in our environment.","container-title":"PLOS Medicine","DOI":"10.1371/journal.pmed.0050219","ISSN":"1549-1676","issue":"11","journalAbbreviation":"PLOS Medicine","language":"en","page":"e219","source":"PLoS Journals","title":"Emergence of Azole Resistance in Aspergillus fumigatus and Spread of a Single Resistance Mechanism","volume":"5","author":[{"family":"Snelders","given":"Eveline"},{"family":"Lee","given":"He nrich A. L.","dropping-particle":"van der"},{"family":"Kuijpers","given":"Judith"},{"family":"Rijs","given":"Anthonius J. M. M."},{"family":"Varga","given":"János"},{"family":"Samson","given":"Robert A."},{"family":"Mellado","given":"Emilia"},{"family":"Donders","given":"A. Rogier T."},{"family":"Melchers","given":"Willem J. G."},{"family":"Verweij","given":"Paul E."}],"issued":{"date-parts":[["2008",11,11]]}}},{"id":2654,"uris":["http://zotero.org/users/local/opHgB1Dy/items/AUHD882B"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/AUHD882B"],"itemData":{"id":2654,"type":"article-journal","abstract":"To the Editor: The use of voriconazole has become common for the management of invasive aspergillosis. However, therapy with voriconazole still sometimes fails, more often because of unresponsive underlying disease than because of resistance of the fungus. Since the first description of itraconazole resistance in Aspergillus fumigatus, 1 three amino acid substitutions in the 14α-sterol demethylase cyp51A gene, which is the target site for azole drugs, have been described.2 Our laboratory receives fungal isolates for identification and susceptibility testing from throughout the Netherlands. Since 2002, using Clinical and Laboratory Standards Institute methodology, we have observed an increase in the number of . . .","container-title":"New England Journal of Medicine","DOI":"10.1056/NEJMc061720","ISSN":"0028-4793","issue":"14","note":"PMID: 17409336","page":"1481-1483","source":"Taylor and Francis+NEJM","title":"Multiple-Triazole–Resistant Aspergillosis","volume":"356","author":[{"family":"Verweij","given":"Paul E."},{"family":"Mellado","given":"Emilia"},{"family":"Melchers","given":"Willem J.G."}],"issued":{"date-parts":[["2007",4,5]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}], with subsequent studies finding that 53%–64% of patients with resistant infection lacked exposure to medical triazoles [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"65A440LW","properties":{"formattedCitation":"(van der Linden et al. 2011, 2013)","plainCitation":"(van der Linden et al. 2011, 2013)","noteIndex":0},"citationItems":[{"id":2251,"uris":["http://zotero.org/users/local/opHgB1Dy/items/AXJVNPXJ"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/AXJVNPXJ"],"itemData":{"id":2251,"type":"article-journal","abstract":"Background. Azole resistance is an emerging problem in Aspergillus fumigatus and complicates the management of patients with Aspergillus-related diseases. Selection of azole resistance may occur through exposure to azole fungicides in the environment. In the Netherlands a surveillance network was used to investigate the epidemiology of resistance selection in A. fumigatus.\nMethods. Clinical A. fumigatus isolates were screened for azole resistance in 8 university hospitals using azole agar dilution plates. Patient information was collected using an online questionnaire and azole-resistant A. fumigatus isolates were analyzed using gene sequencing, susceptibility testing, and genotyping. Air sampling was performed to investigate the presence of resistant isolates in hospitals and domiciles.\nResults. Between December 2009 and January 2011, 1315 A. fumigatus isolates from 921 patients were screened. A new cyp51A-mediated resistance mechanism (TR46/Y121F/T289A) was observed in 21 azole-resistant isolates from 15 patients in 6 hospitals. TR46/Y121F/T289A isolates were highly resistant to voriconazole (minimum inhibitory concentration ≥16 mg/L). Eight patients presented with invasive aspergillosis due to TR46/Y121F/T289A, and treatment failed in all 5 patients receiving primary therapy with voriconazole. TR46/Y121F/T289A Aspergillus fumigatus was recovered from 6 of 10 sampled environmental sites.\nConclusions. We describe the emergence and geographical migration of a voriconazole highly resistant A. fumigatus that was associated with voriconazole treatment failure in patients with invasive aspergillosis. Recovery of TR46/Y121F/T289A from the environment suggests an environmental route of resistance selection. Exposure of A. fumigatus to azole fungicides may facilitate the emergence of new resistance mechanisms over time, thereby compromising the use of azoles in the management of Aspergillus-related diseases.","container-title":"Clinical Infectious Diseases","DOI":"10.1093/cid/cit320","ISSN":"1537-6591, 1058-

4838","issue":"4","language":"en","page":"513-520","source":"DOI.org (Crossref)","title":"Aspergillosis due to Voriconazole Highly Resistant Aspergillus fumigatus and Recovery of Genetically Related Resistant Isolates From Domiciles","volume":"57","author":[{"family":"Linden","given":"Jan W. M.","non-dropping-particle":"van der"},{"family":"Camps","given":"Simone M. T."},{"family":"Kampinga","given":"Greetje A."},{"family":"Arends","given":"Jan P. A."},{"family":"Debets-Ossenkopp","given":"Yvette J."},{"family":"Haas","given":"Pieter J. A."},{"family":"Rijnders","given":"Bart J. A."},{"family":"Kuijper","given":"Ed J."},{"family":"Tiel","given":"Frank H.","non-dropping-particle":"van"},{"family":"Varga","given":"János"},{"family":"Karawajczyk","given":"Anna"},{"family":"Zoll","given":"J."},{"family":"Melchers","given":"Willem J. G."},{"family":"Verweij","given":"Paul E."}],"issued":{"date-parts":[["2013",8,15]]}},{"id":2257,"uris":["http://zotero.org/users/local/opHgB1Dy/items/4E2AR8VY"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/4E2AR8VY"],"itemData":{"id":2257,"type":"article-journal","abstract":"The prevalence and spread of azole resistance in clinical Aspergillus fumigatus isolates in the Netherlands are currently unknown. Therefore, we perfo...","DOI":"10.3201/eid1710.110226","language":"en-us","source":"wwwnc.cdc.gov","title":"Clinical Implications of Azole Resistance in Aspergillus fumigatus, the Netherlands, 2007–2009 - Volume 17, Number 10—October 2011 - Emerging Infectious Diseases journal - CDC","URL":"https://wwwnc.cdc.gov/eid/article/17/10/11-0226_article","author":[{"family":"Linden","given":"Jan W. M.","non-dropping-particle":"van der"},{"family":"Snelders","given":"Eveline"},{"family":"Kampinga","given":"Greetje A."},{"family":"Rijnders","given":"Bart J. A."},{"family":"Mattsson","given":"Eva"},{"family":"Debets-Ossenkopp","given":"Yvette J."},{"family":"Kuijper","given":"Ed J."},{"family":"Tiel","given":"Frank H. Van"},{"family":"Melchers","given":"Willem J. G."},{"family":"Verweij","given":"Paul

E."}],"accessed":{"date-parts":[["2019",7,20]]},"issued":{"date-parts":[["2011"]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}].

Because triazoles are widely used in agriculture as fungicides, researchers suspected that the TR_{34} -based resistance developed in the environment under fungicide-induced selection pressure [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"zLRY56Ra","properties":{"formattedCitation":"(Bromley et al. 2014; Snelders et al. 2009)","plainCitation":"(Bromley et al. 2014; Snelders et al. 2009)","noteIndex":0},"citationItems":[{"id":2650,"uris":["http://zotero.org/users/local/opHgB1Dy/items/XWRQW36L"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/XWRQW36L"],"itemData":{"id":2650,"type":"article-journal","container-title":"Applied and Environmental Microbiology","DOI":"10.1128/AEM.00231-09","ISSN":"0099-2240","issue":"12","journalAbbreviation":"Applied and Environmental Microbiology","language":"en","page":"4053-4057","source":"DOI.org (Crossref)","title":"Possible Environmental Origin of Resistance of Aspergillus fumigatus to Medical Triazoles","volume":"75","author":[{"family":"Snelders","given":"E."},{"family":"Huis in 't Veld","given":"R. A. G."},{"family":"Rijs","given":"A. J. M. M."},{"family":"Kema","given":"G. H. J."},{"family":"Melchers","given":"W. J. G."},{"family":"Verweij","given":"P. E."}],"issued":{"date-parts":[["2009",6,15]]}}},{"id":2205,"uris":["http://zotero.org/users/local/opHgB1Dy/items/JFC34DIK"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/JFC34DIK"],"itemData":{"id":2205,"type":"article-journal","abstract":"The aim of this study was to survey environmental isolates of Aspergillus resistant to azoles in azole-treated and naïve areas to determine whether resistance could be related to azole treatment history. Aspergillus fumigatus was sampled from the centre of a large city and from fields with known azole history. Azole resistance was determined and sequencing was performed to identify strains and mutations in the cyp51A gene. Azole resistance was detected in azole-treated field isolates but not in urban isolates (P=0.038). In addition, an azole-resistant isolate of Neosartorya fischeri was isolated. These results support the hypothesis that agricultural azole use may lead to resistance in environmental fungi of clinical importance. We report the first environmental UK TR34/L98H isolate of A. fumigatus.","container-title":"Journal of Global Antimicrobial Resistance","DOI":"10.1016/j.jgar.2014.05.004","ISSN":"2213-7165","issue":"4","journalAbbreviation":"Journal of Global Antimicrobial Resistance","page":"276-279","source":"ScienceDirect","title":"Occurrence of azole-resistant species of Aspergillus in the UK environment","volume":"2","author":[{"family":"Bromley","given":"Michael J."},{"family":"Muijlwijk","given":"Guus","non-dropping-particle":"van"},{"family":"Fraczek","given":"Marcin G."},{"family":"Robson","given":"Geoff"},{"family":"Verweij","given":"Paul E."},{"family":"Denning","given":"David W."},{"family":"Bowyer","given":"Paul"}],"issued":{"date-parts":[["2014",12,1]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}] and that infections resulted from exposure to already-resistant *A. fumigatus* rather than resistance developing in the patient [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"H4MUiThX","properties":{"formattedCitation":"(Berger et al. 2017)","plainCitation":"(Berger et al. 2017)","noteIndex":0},"citationItems":[{"id":2231,"uris":["http://zotero.org/users/local/opHgB1Dy/items/KV9EE5TK"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/KV9EE5TK"],"itemData":{"id":2231,"type":"article-journal","abstract":"Agricultural industry uses pesticides to optimize food production for the growing human population. A major issue for crops is fungal phytopathogens, which are treated mainly with azole fungicides. Azoles are also the main medical treatment in the management of Aspergillus diseases caused by ubiquitous fungi, such as Aspergillus fumigatus. However, epidemiological research demonstrated an increasing prevalence of azole-resistant strains in A. fumigatus. The main resistance mechanism is a combination of alterations in the gene cyp51A (TR34/L98H). Surprisingly, this mutation is not only found in patients receiving long-term azole therapy for chronic aspergillosis but also in azole naïve patients. This suggests an environmental route of resistance through the exposure of azole fungicides in agriculture. In this review, we report data from several studies that strongly suggest that agricultural azoles are responsible for medical treatment failure in azole-naïve patients in clinical settings.","container-title":"Frontiers in Microbiology","DOI":"10.3389/fmicb.2017.01024","ISSN":"1664-302X","journalAbbreviation":"Front Microbiol","note":"PMID: 28638374\nPMCID: PMC5461301","source":"PubMed Central","title":"Azole Resistance in Aspergillus fumigatus: A Consequence of Antifungal Use in Agriculture?","title-short":"Azole Resistance in Aspergillus fumigatus","URL":"https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5461301/","volume":"8","author":[{"family":"Berger","given":"Sarah"},{"family":"El Chazli","given":"Yassine"},{"family":"Babu","given":"Ambrin F."},{"family":"Coste","given":"Alix T."}],"accessed":{"date-parts":[["2019",7,20]]},"issued":{"date-parts":[["2017",6,7]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}]. Subsequent research provided additional evidence for this hypothesis and identified a second genotype, TR_{46}/Y121F/T289A (TR_{46}), thought to be linked to fungicide use [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"D8ig3lvS","properties":{"formattedCitation":"(Astvad et al. 2014; Chowdhary et al. 2014b, 2015; Lavergne et al. 2015; Le Pape et al. 2016; Montesinos et al. 2014; Steinmann et al. 2015; van der Linden et al. 2013, 2015; Vermeulen et al. 2012)","plainCitation":"(Astvad et al. 2014; Chowdhary et al. 2014b, 2015; Lavergne et al. 2015; Le Pape et al. 2016; Montesinos et al. 2014; Steinmann et al. 2015; van der Linden et al. 2013, 2015; Vermeulen et al. 2012)","noteIndex":0},"citationItems":[{"id":2312,"uris":["http://zotero.org/users/local/opHgB1Dy/item s/ILQ5GLU4"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/ILQ5GLU4"],"itemData":{"id":2312,"type":"article-journal","abstract":"Azole resistance in Aspergillus fumigatus is an emerging public health concern. Recently, a novel fungicide-driven mutation in the cyp51A gene and its promoter, TR46/Y121F/T289A, leading to high-level resistance to voriconazole has been identified in The Netherlands, Belgium, Germany, Denmark, Tanzania, and India in both clinical and environmental samples. Here we report the first description of A. fumigatus carrying this mutation in France, in a cystic fibrosis patient, underlining the need for extensive monitoring of Aspergillus resistance.","container-title":"Antimicrobial Agents and Chemotherapy","DOI":"10.1128/AAC.00127-15","ISSN":"1098-6596","issue":"7","journalAbbreviation":"Antimicrob. Agents Chemother.","language":"eng","note":"PMID: 25918139\nPMCID: PMC4468656","page":"4331-4335","source":"PubMed","title":"First description of azole-resistant Aspergillus fumigatus due to TR46/Y121F/T289A mutation in France","volume":"59","author":[{"family":"Lavergne","given":"Rose-Anne"},{"family":"Morio","given":"Florent"},{"family":"Favennec","given":"Loïc"},{"family":"Dominique","given":"Stéphane"},{"family":"Meis","given":"Jacques F."},{"family":"Gargala","given":"Gilles"},{"family":"Verweij","given":"Paul E."},{"family":"Le Pape","given":"Patrice"}],"issued":{"date-parts":[["2015",7]]}}},{"id":2794,"uris":["http://zotero.org/users/local/opHgB1Dy/items/R55AQP92"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/R55AQP92"],"itemData":{"id":2794,"type":"article-journal","abstract":"A new azole resistance mechanism in Aspergillus fumigatus consisting of a TR46/Y121F/T289A alteration in the cyp51A gene was recently described in the Netherlands. Strains containing these mutations are associated with invasive infection and therapy failure. This communication describes the first case of fatal invasive aspergillosis caused by TR46/Y121F/T289A outside the Netherlands, in the neighboring country of Belgium, suggesting geographical spread. TR46/Y121F/T289A leads to a recognisable phenotypic susceptibility pattern which should trigger cyp51A genotyping to monitor further spread.","container-title":"Eurosurveillance","DOI":"10.2807/ese.17.48.20326-en","ISSN":"1560-7917","issue":"48","language":"en","page":"20326","source":"www.eurosurveillance.org","title":"Azole-resistant Aspergillus fumigatus due to TR46/Y121F/T289A mutation emerging in Belgium, July 2012","volume":"17","author":[{"family":"Vermeulen","given":"E."},{"family":"Maertens","given":"J."},{"family":"Schoemans","given":"H."},{"family":"Lagrou","given":"K."}],"issued":{"date-parts":[["2012",11,29]]}}},{"id":2251,"uris":["http://zotero.org/users/local/opHgB1Dy/items/AXJVNPXJ"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/AXJVNPXJ"],"itemData":{"id":2251,"type":"article-journal","abstract":"Background. Azole resistance is an emerging problem in Aspergillus fumigatus and complicates the management of patients with Aspergillus-related diseases. Selection of azole resistance may occur through exposure to azole fungicides in the environment. In the Netherlands a surveillance network was used to investigate the epidemiology of resistance selection in A. fumigatus.\nMethods. Clinical A. fumigatus isolates were screened for azole resistance in 8 university hospitals using azole agar dilution plates. Patient information was collected using an online questionnaire and azole-resistant A. fumigatus isolates were analyzed using gene sequencing, susceptibility testing, and genotyping. Air sampling was performed to investigate the presence of resistant isolates in hospitals and domiciles.\nResults. Between December 2009 and January 2011, 1315 A. fumigatus isolates from 921 patients were screened. A new cyp51A-mediated resistance mechanism (TR46/Y121F/T289A) was observed in 21 azole-resistant isolates from 15 patients in 6 hospitals. TR46/Y121F/T289A isolates were highly resistant to voriconazole (minimum inhibitory concentration ≥16 mg/L). Eight patients presented with invasive aspergillosis due to TR46/Y121F/T289A, and treatment failed in all 5 patients receiving primary therapy with voriconazole. TR46/Y121F/T289A Aspergillus fumigatus was recovered from 6 of 10 sampled environmental sites.\nConclusions. We describe the emergence and geographical migration of a voriconazole highly resistant A. fumigatus that was associated with voriconazole treatment failure in patients with invasive aspergillosis. Recovery of TR46/Y121F/T289A from the environment suggests an environmental route of resistance selection. Exposure of A. fumigatus to azole fungicides may facilitate the emergence of new resistance mechanisms over time, thereby compromising the use of azoles in the management of Aspergillus-related diseases.","container-title":"Clinical Infectious Diseases","DOI":"10.1093/cid/cit320","ISSN":"1537-6591, 1058-4838","issue":"4","language":"en","page":"513-520","source":"DOI.org (Crossref)","title":"Aspergillosis due to Voriconazole Highly Resistant Aspergillus fumigatus and Recovery of Genetically Related Resistant Isolates From Domiciles","volume":"57","author":[{"family":"Linden","given":"Jan W. M.","non-dropping-particle":"van der"},{"family":"Camps","given":"Simone M. T."},{"family":"Kampinga","given":"Greetje A."},{"family":"Arends","given":"Jan P. A."},{"family":"Debets-Ossenkopp","given":"Yvette J."},{"family":"Haas","given":"Pieter J. A."},{"family":"Rijnders","given":"Bart J. A."},{"family":"Kuijper","given":"Ed J."},{"family":"Tiel","given":"Frank H.","non-dropping-particle":"van"},{"family":"Varga","given":"János"},{"family":"Karawajczyk","given":"Anna"},{"family":"Zoll","given":"J."},{"family":"Melchers","given":"Willem J. G."},{"family":"Verweij","given":"Paul E."}],"issued":{"date-parts":[["2013",8,15]]}},{"id":2194,"uris":["http://zotero.org/users/local/opHgB1Dy/items/Q5ICJ6WJ"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/Q5ICJ6WJ"],"itemData":{"id":2194,"type":"article-journal","abstract":"Azole-resistant Aspergillus fumigatus harboring the TR34/L98H or TR46/Y121F/T289A alterations is increasingly found in Europe and Asia. Here, we present the first clinical cases of TR46/Y121/T289A and three cases of TR34/L98H outside the cystic fibrosis (CF) population in Denmark and the results of environmental surveys. Four patients (2012 to 2014) with 11 A. fumigatus and 4 Rhizomucor pusillus isolates and 239 soil samples (spring 2010 and autumn 2013, respectively) with a total of 113 A. fumigatus isolates were examined. Aspergillus isolates were screened for azole resistance using azole-containing agar. Confirmatory susceptibility testing was done using the EUCAST microbroth dilution EDEF 9.1 reference method. For relevant A. fumigatus isolates, CYP51A sequencing and microsatellite genotyping were performed. Three patients harbored TR34/L98H isolates. Two were azole naive at the time of acquisition and two were coinfected with wild-type A. fumigatus or R. pusillus isolates, complicating and delaying diagnosis. The TR46/Y121F/T289A strain was isolated in 2014 from a lung transplant patient. Genotyping indicated that susceptible and resistant Aspergillus isolates were unrelated and that no transmission between patients occurred. Azole resistance was not detected in any of the 113 soil isolates. TR34/L98H and TR46/Y121F/T289A alterations appear to be emerging in the clinical setting in Denmark and now involve azole-naive patients. Two recent soil-sampling surveys in Denmark were unable to indicate any increased prevalence of azole-resistant A. fumigatus in the environment. These findings further support the demand for real-time susceptibility testing of all clinically relevant isolates and for studies investigating the seasonal variation and ecological niches for azole-resistant environmental A. fumigatus.","container-title":"Antimicrobial Agents and Chemotherapy","DOI":"10.1128/AAC.02855-14","ISSN":"0066-4804","issue":"9","journalAbbreviation":"Antimicrob Agents Chemother","note":"PMID: 24936595\nPMCID: PMC4135837","page":"5096-5101","source":"PubMed Central","title":"First Detection of TR46/Y121F/T289A and TR34/L98H Alterations in Aspergillus fumigatus Isolates from Azole-Naive Patients in Denmark despite Negative Findings in the Environment","volume":"58","author":[{"family":"Astvad","given":"K. M. T."},{"family":"Jensen","given":"R. H."},{"family":"Hassan","given":"T. M."},{"family":"Mathiasen","given":"E. G."},{"family":"Thomsen","given":"G. M."},{"family":"Pedersen","given":"U. G."},{"family":"Christensen","given":"M."},{"family":"Hilberg","given":"O."},{"family":"Arendrup","given":"M. C."}],"issued":{"date-parts":[["2014",9]]}},{"id":2801,"uris":["http://zotero.org/users/local/opHgB1Dy/items/AP9XEHE6"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/AP9XEHE6"],"itemData":{"id":2801,"type":"article-journal","abstract":"AbstractObjectives. Aspergillus fumigatus is the most common agent of invasive aspergillosis (IA). In recent years, resistance to triazoles, the mainstay of IA","container-title":"Journal of Antimicrobial Chemotherapy","DOI":"10.1093/jac/dku566","ISSN":"0305-7453","issue":"5","journalAbbreviation":"J Antimicrob Chemother","language":"en","page":"1522-1526","source":"academic.oup.com","title":"Emergence of azole-resistant invasive aspergillosis in HSCT recipients in Germany","volume":"70","author":[{"family":"Steinmann","given":"J."},{"family":"Hamprecht","given":"A."},{"family":"Vehreschild","given":"M. J. G. T."},{"family":"Cornely","given":"O. A."},{"family":"Buchheidt","given":"D."},{"family":"Spiess","given":"B."},{"family":"Koldehoff","given":"M."},{"family":"Buer","given":"J."},{"family":"Meis","given":"J. F."},{"family":"Rath","given":"P.-M."}],"issued":{"date-parts":[["2015",5,1]]}},{"id":2798,"uris":["http://zotero.org/users/local/opHgB1Dy/items/TTJBEPFU"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/TTJBEPFU"],"itemData":{"id":2798,"type":"article-journal","abstract":"Sir,","container-title":"Journal of Antimicrobial Chemotherapy","DOI":"10.1093/jac/dku289","ISSN":"0305-7453","issue":"12","journalAbbreviation":"J Antimicrob Chemother","language":"en","page":"3439-3440","source":"academic.oup.com","title":"New case of azole-resistant Aspergillus fumigatus due to TR46/Y121F/T289A mutation in Belgium","volume":"69","author":[{"family":"Montesinos","given":"Isabel"},{"family":"Dodemont","given":"Magali"},{"family":"Lagrou","given":"Katrien"},{"family":"Jacobs","given":"Frederique"},{"family":"Etienne","given":"Isabelle"},{"family":"Denis","given":"Olivier"}],"issued":{"date-parts":[["2014",12,1]]}},{"id":2806,"uris":["http://zotero.org/users/local/opHgB1Dy/items/ATYYTHBB"], "uri":["http://zotero.org/users/local/opHgB1Dy/items/ATYYTHBB"],"itemData":{"id":2806,"type":"article-journal","abstract":"AbstractObjectives. Azole resistance in Aspergillus fumigatus isolates has been increasingly reported with variable prevalence worldwide and is challenging the","container-title":"Journal of Antimicrobial Chemotherapy","DOI":"10.1093/jac/dku259","ISSN":"0305-7453","issue":"11","journalAbbreviation":"J Antimicrob Chemother","language":"en","page":"2979-2983","source":"academic.oup.com","title":"Multi-azole-resistant Aspergillus fumigatus in the environment in Tanzania","volume":"69","author":[{"family":"Chowdhary","given":"Anuradha"},{"family":"Sharma","given":"Cheshta"},{"family":"Boom","given":"Mara","non-dropping-particle":"van den"},{"family":"Yntema","given":"Jan Bart"},{"family":"Hagen","given":"Ferry"},{"family":"Verweij","given":"Paul E."},{"family":"Meis","given":"Jacques F."}],"issued":{"date-parts":[["2014",11,1]]}}},{"id":2278,"uris":["http://zotero.org/users/local/opHgB1Dy/items/8G8DKIX9"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/8G8DKIX9"],"itemData":{"id":2278,"type":"article-journal","abstract":"Aspergillus fumigatus causes varied clinical syndromes ranging from colonization to deep infections. The mainstay of therapy of Aspergillus diseases is triazoles but several studies globally highlighted variable prevalence of triazole resistance, which hampers the management of aspergillosis. We studied the prevalence of resistance in clinical A. fumigatus isolates during 4 years in a referral Chest Hospital in Delhi, India and reviewed the scenario in Asia and the Middle East. Aspergillus species (n=2117) were screened with selective plates for azole resistance. The isolates included 45.4% A. flavus, followed by 32.4% A. fumigatus, 15.6% Aspergillus species and 6.6% A. terreus. Azole resistance was found in only 12 (1.7%) A. fumigatus isolates.These triazole resistant A. fumigatus (TRAF) isolates were subjected to (a) calmodulin and β tubulin gene sequencing (b) in vitro antifungal susceptibility testing against triazoles using CLSI M38-A2 (c) sequencing of cyp51A gene and real-time PCR assay for detection of mutations and (d) microsatellite typing of the resistant isolates. TRAF harbored TR34/L98H mutation in 10 (83.3%) isolates with a pan-azole resistant phenotype. Among the remaining 2 TRAF isolates, one had G54E and the other had three non-synonymous point mutations. The majority of patients were diagnosed as invasive aspergillosis followed by allergic bronchopulmonary aspergillosis and chronic pulmonary aspergillosis. The Indian TR34/L98H isolates had a unique genotype and were distinct from the Chinese, Middle East and European TR34/L98H strains. This resistance mechanism has been linked to the use of fungicide azoles in agricultural practices in Europe as it has been mainly reported from azole naïve patients. Reports published from Asia demonstrate the same environmental resistance mechanism in A. fumigatus isolates from two highly populated countries in Asia i.e., China and India and also from the neighboring Middle East.","container-title":"Frontiers in Microbiology","DOI":"10.3389/fmicb.2015.00428","ISSN":"1664-302X","journalAbbreviation":"Front. Microbiol.","language":"English","source":"Frontiers","title":"Prevalence and mechanism of triazole resistance in Aspergillus fumigatus in a referral chest hospital in Delhi, India and an update of the situation in Asia","URL":"https://www.frontiersin.org/articles/10.3389/fmicb.2015.00428/full","volume":"6","author":[{"family":"Chowdhary","given":"Anuradha"},{"family":"Sharma","given":"Cheshta"},{"family":"Kathuria","given":"Shallu"},{"family":"Hagen","given":"Ferry"},{"family":"Meis","given":"Jacques F."}],"accessed":{"date-parts":[["2019",7,20]]},"issued":{"date-parts":[["2015"]]}}},{"id":2476,"uris":["http://zotero.org/users/local/opHgB1Dy/items/R8M6S9I6"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/R8M6S9I6"],"itemData":{"id":2476,"type":"article-journal","container-title":"Emerging Infectious Diseases","DOI":"10.3201/eid2201.150978","ISSN":"1080-6040","issue":"1","journalAbbreviation":"Emerg Infect Dis","note":"PMID: 26690795\nPMCID: PMC4696698","page":"156-157","source":"PubMed Central","title":"Multiple Fungicide-Driven

Alterations in Azole-Resistant Aspergillus fumigatus, Colombia, 2015","volume":"22","author":[{"family":"Le Pape","given":"Patrice"},{"family":"Lavergne","given":"Rose-Anne"},{"family":"Morio","given":"Florent"},{"family":"Alvarez-Moreno","given":"Carlos"}],"issued":{"date-parts":[["2016",1]]}}},{"id":253,"uris":["http://zotero.org/users/local/opHgB1Dy/items/6KYEUN44"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/6KYEUN44"],"itemData":{"id":253,"type":"article-journal","abstract":"To investigate azole resistance in clinical Aspergillus isolates, we conducted prospective multicenter international surveillance. A total of 3,788 Aspergillus isolates were screened in 22 centers from 19 countries. Azole-resistant A. fumigatus was more frequently found (3.2% prevalence) than previously acknowledged, causing resistant invasive and noninvasive aspergillosis and severely compromising clinical use of azoles.","container-title":"Emerging Infectious Diseases","DOI":"10.3201/eid2106.140717","ISSN":"1080-6040","issue":"6","journalAbbreviation":"Emerg Infect Dis","note":"PMID: 25988348\nPMCID: PMC4451897","page":"1041-1044","source":"PubMed Central","title":"Prospective Multicenter International Surveillance of Azole Resistance in Aspergillus fumigatus","volume":"21","author":[{"family":"Linden","given":"J.W.M.","non-dropping-particle":"van der"},{"family":"Arendrup","given":"M.C."},{"family":"Warris","given":"A."},{"family":"Lagrou","given":"K."},{"family":"Pelloux","given":"H."},{"family":"Hauser","given":"P.M."},{"family":"Chryssanthou","given":"E."},{"family":"Mellado","given":"E."},{"family":"Kidd","given":"S.E."},{"family":"Tortorano","given":"A.M."},{"family":"Dannaoui","given":"E."},{"family":"Gaustad","given":"P."},{"family":"Baddley","given":"J.W."},{"family":"Uekötter","given":"A."},{"family":"Lass-Flörl","given":"C."},{"family":"Klimko","given":"N."},{"family":"Moore","given":"C.B."},{"family":"Denning","given":"D.W."},{"family":"Pasqualotto","given":"A.C."},{"family":"Kibbler","given":"C."},{"family":"Ari kan-Akdagli","given":"S."},{"family":"Andes","given":"D."},{"family":"Meletiadis","given":"J."},{"family":"Naumiuk","given":"L."},{"family":"Nucci","given":"M."},{"family":"Melchers","given":"W.J.G."},{"family":"Verweij","given":"P.E."}],"issued":{"date-parts":[["2015",6]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}]. Although the TR-based mechanisms may not be a definitive marker of environmental resistance — one report described a resistant isolate with a TR$_{120}$ mechanism in a patient on long-term triazole therapy for chronic aspergillosis [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"oHk6jQEX","properties":{"formattedCitation":"(Hare et al. 2019)","plainCitation":"(Hare et al. 2019)","noteIndex":0},"citationItems":[{"id":3058,"uris":["http://zotero.org/users/local/opHgB1Dy/items/CK4B6HJD"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/CK4B6HJD"],"itemData":{"id":3058,"type":"article-journal","abstract":"We report a fatal aspergillosis case in which STRAf typing and whole-genome sequencing substantiated in vivo emergence of an azole-resistant Aspergill...","DOI":"10.3201/eid2503.180297","language":"en-us","source":"wwwnc.cdc.gov","title":"In Vivo Selection of a Unique Tandem Repeat Mediated Azole Resistance Mechanism (TR120) in Aspergillus fumigatus cyp51A, Denmark - Volume 25, Number 3—March 2019 - Emerging Infectious Diseases journal - CDC","URL":"https://wwwnc.cdc.gov/eid/article/25/3/18-0297_article","author":[{"family":"Hare","given":"Rasmus K."},{"family":"Gertsen","given":"Jan B."},{"family":"Astvad","given":"Karen M. T."},{"family":"Degn","given":"Kristine B."},{"family":"Løkke","given":"Anders"},{"family":"Stegger","given":"Marc"},{"family":"Andersen","given":"Paal S."},{"family":"Kristensen","given":"Lise"},{"family":"Arendrup","given":"Maiken C."}],"accessed":{"date-parts":[["2019",10,22]]},"issued":{"date-parts":[["2019"]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}] — overall evidence suggests that isolates with TR$_{34}$ and TR$_{46}$ mutations result from environmental triazole exposure [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"qXNfozQV","properties":{"formattedCitation":"(Buil et al. 2019)","plainCitation":"(Buil et al. 2019)","noteIndex":0},"citationItems":[{"id":2886,"uris":["http://zotero.org/users/local/opHgB1Dy/items/4VJ3ZWSG"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/4VJ3ZWSG"],"itemData":{"id":2886,"type":"article-journal","container-title":"PLOS Pathogens","DOI":"10.1371/journal.ppat.1007858","ISSN":"1553-7374","issue":"8","journalAbbreviation":"PLOS Pathogens","language":"en","page":"e1007858","source":"PLoS Journals","title":"The fading boundaries between patient and environmental routes of triazole resistance selection in Aspergillus fumigatus","volume":"15","author":[{"family":"Buil","given":"Jochem B."},{"family":"Hare","given":"Rasmus K."},{"family":"Zwaan","given":"Bas J."},{"family":"Arendrup","given":"Maiken C."},{"family":"Melchers","given":"Willem J. G."},{"family":"Verweij","given":"Paul E."}],"issued":{"date-parts":[["2019",8,22]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}].

TR_{34} and TR_{46}-mediated resistance has become common in patients with aspergillosis in parts of Europe, where up to 20% of infections are now resistant to triazoles [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"SZk1RqEP","properties":{"formattedCitation":"(Bueid et al. 2010; Leli\\uc0\\u232{}vre et al. 2013; Resendiz-Sharpe et al. 2019; van der Linden et al. 2015)","plainCitation":"(Bueid et al. 2010; Lelièvre et al. 2013; Resendiz-Sharpe et al. 2019; van der Linden et al. 2015)","noteIndex":0},"citationItems":[{"id":3040,"uris":["http://zotero.org/users/local/opHgB1Dy/items/YC9H5WZR"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/YC9H5WZR"],"itemData":{"id":30

40,"type":"article-journal","abstract":"AbstractObjectives. Resistance to azole antifungal drugs in Aspergillus fumigatus is now a major clinical problem in some locations. Here we update our previou","container-title":"Journal of Antimicrobial Chemotherapy","DOI":"10.1093/jac/dkq279","ISSN":"0305-7453","issue":"10","journalAbbreviation":"J Antimicrob Chemother","language":"en","page":"2116-2118","source":"academic.oup.com","title":"Azole antifungal resistance in Aspergillus fumigatus: 2008 and 2009","title-short":"Azole antifungal resistance in Aspergillus fumigatus","volume":"65","author":[{"family":"Bueid","given":"Ahmed"},{"family":"Howard","given":"Susan J."},{"family":"Moore","given":"Caroline B."},{"family":"Richardson","given":"Malcolm D."},{"family":"Harrison","given":"Elizabeth"},{"family":"Bowyer","given":"Paul"},{"family":"Denning","given":"David W."}],"issued":{"date-parts":[["2010",10,1]]}}},{"id":3037,"uris":["http://zotero.org/users/local/opHgB1Dy/items/7GAM2CCR"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/7GAM2CCR"],"itemData":{"id":3037,"type":"article-journal","abstract":"Azole resistance has appeared recently in Aspergillus fumigatus and increased dangerously in the last decade. The main resistance mechanism is a point mutation of CYP51A, the gene encoding 14α-sterol demethylase, the target enzyme of azole antifungal drugs. This mutation can induce resistance to itraconazole alone or multi-azole resistance. CYP51A mutation can occur in two cases. The first usually concerns patients receiving long-term azole therapy, most of the time for chronic aspergillosis, and involves a wide range of mutations. The second is due to the use of azole fungicides in agriculture. The latter favors a single mutagenesis event: a substitution of leucine for histidine at codon 98 and the tandem repeat of a 34-base pair tandem sequence in the CYP51A gene promoter region. This confers cross-resistance to all azole antifungal drugs. This emerging and environmentally linked issue is of growing concern for the management of antifungal therapy. This mechanism of resistance was first described in the Netherlands and is now reported worldwide. It may have become the leading mechanism of azole resistance in A. fumigatus. Azoles are major agents for the treatment of aspergillosis, and the only oral antifungals. Infection with antifungal-resistant strains is correlated with treatment failure. This emerging phenomenon stresses the urgent need for new preventive strategies (controlled use of antifungals and azole prophylaxis), new diagnostic strategies (early detection of resistance), and new therapeutic strategies in the management of A. fumigatus infections.\nRésumé\nLa résistance d’Aspergillus fumigatus aux azolés est apparue récemment et est en dangereuse augmentation depuis dix ans. Son principal mécanisme est lié à la mutation du gène CYP51A codant pour l’enzyme 14-α-déméthylase, cible des azolés. Celle-ci peut conduire à une résistance touchant l’itraconazole seul ou l’ensemble des molécules triazolées. Deux situations peuvent engendrer des mutations du gène CYP51A. La première apparaît classiquement chez le patient traité par azolés au long cours – le plus souvent pour une aspergillose chronique – et fait intervenir une grande variété de mutations. La seconde, émergente, est due à l’utilisation de fongicides azolés en agriculture et confère une résistance croisée à tous les azolés. Cette dernière repose sur la substitution d’une leucine par l’histidine au codon 98 du gène CYP51A, associée à la répétition en tandem d’une séquence de 34 paires de bases dans la région promotrice du gène (TR/L98H). Initialement décrit aux Pays-Bas, ce type de résistance diffuse maintenant à travers le monde et pourrait même être devenu, la principale source de résistance d’A. fumigatus aux azolés. Les azolés tiennent une place majeure dans le traitement des infections aspergillaires et représentent la seule classe utilisable par voie orale. Une infection à isolat résistant étant directement associée à l’échec thérapeutique, l’émergence de ce phénomène implique la mise en place de nouvelles stratégies à la fois préventives (maîtrise de l’utilisation des fongicides et des prophylaxies par azolés), diagnostiques (détection précoce de la résistance), et thérapeutiques dans la gestion des infections à A. fumigatus.","container-title":"Médecine et Maladies Infectieuses","DOI":"10.1016/j.medmal.2013.02.010","ISSN":"0399-077X","issue":"4","journalAbbreviation":"Médecine et Maladies

Infectieuses","language":"en","page":"139-145","source":"ScienceDirect","title":"Azole resistant Aspergillus fumigatus: An emerging problem","title-short":"Azole resistant Aspergillus fumigatus","volume":"43","author":[{"family":"Lelièvre","given":"L."},{"family":"Groh","given":"M."},{"family":"Angebault","given":"C."},{"family":"Maherault","given":"A. -C."},{"family":"Didier","given":"E."},{"family":"Bougnoux","given":"M. -E."}],"issued":{"date-parts":[["2013",4,1]]}}},{"id":3032,"uris":["http://zotero.org/users/local/opHgB1Dy/items/2M7K6H7Y"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/2M7K6H7Y"],"itemData":{"id":3032,"type":"article-journal","abstract":"AbstractBackground. Increasing resistance of Aspergillus fumigatus to triazoles in high-risk populations is a concern. Its impact on mortality is not well unde","container-title":"Journal of Antimicrobial Chemotherapy","DOI":"10.1093/jac/dkz258","ISSN":"0305-7453","issue":"9","journalAbbreviation":"J Antimicrob Chemother","language":"en","page":"2759-2766","source":"academic.oup.com","title":"Prevalence of voriconazole-resistant invasive aspergillosis and its impact on mortality in haematology patients","volume":"74","author":[{"family":"Resendiz-Sharpe","given":"Agustin"},{"family":"Mercier","given":"Toine"},{"family":"Lestrade","given":"Pieter P. A."},{"family":"Beek","given":"Martha T.","non-dropping-particle":"van der"},{"family":"Borne","given":"Peter A.","non-dropping-particle":"von dem"},{"family":"Cornelissen","given":"Jan J."},{"family":"De Kort","given":"Elizabeth"},{"family":"Rijnders","given":"Bart J. A."},{"family":"Schauwvlieghe","given":"Alexander F. A. D."},{"family":"Verweij","given":"Paul E."},{"family":"Maertens","given":"Johan"},{"family":"Lagrou","given":"Katrien"}],"issued":{"date-parts":[["2019",9,1]]}}},{"id":253,"uris":["http://zotero.org/users/local/opHgB1Dy/items/6KYEUN44"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/6KYEUN44"],"itemData":{"id":253,"type":"article-journal","abstract":"To investigate azole resistance in clinical Aspergillus isolates, we conducted prospective multicenter international surveillance. A total of 3,788 Aspergillus isolates were screened in

22 centers from 19 countries. Azole-resistant A. fumigatus was more frequently found (3.2% prevalence) than previously acknowledged, causing resistant invasive and noninvasive aspergillosis and severely compromising clinical use of azoles.","container-title":"Emerging Infectious Diseases","DOI":"10.3201/eid2106.140717","ISSN":"1080-6040","issue":"6","journalAbbreviation":"Emerg Infect Dis","note":"PMID: 25988348\nPMCID: PMC4451897","page":"1041-1044","source":"PubMed Central","title":"Prospective Multicenter International Surveillance of Azole Resistance in Aspergillus fumigatus","volume":"21","author":[{"family":"Linden","given":"J.W.M.","non-dropping-particle":"van der"},{"family":"Arendrup","given":"M.C."},{"family":"Warris","given":"A."},{"family":"Lagrou","given":"K."},{"family":"Pelloux","given":"H."},{"family":"Hauser","given":"P.M."},{"family":"Chryssanthou","given":"E."},{"family":"Mellado","given":"E."},{"family":"Kidd","given":"S.E."},{"family":"Tortorano","given":"A.M."},{"family":"Dannaoui","given":"E."},{"family":"Gaustad","given":"P."},{"family":"Baddley","given":"J.W."},{"family":"Uekötter","given":"A."},{"family":"Lass-Flörl","given":"C."},{"family":"Klimko","given":"N."},{"family":"Moore","given":"C.B."},{"family":"Denning","given":"D.W."},{"family":"Pasqualotto","given":"A.C."},{"family":"Kibbler","given":"C."},{"family":"Arikan-Akdagli","given":"S."},{"family":"Andes","given":"D."},{"family":"Meletiadis","given":"J."},{"family":"Naumiuk","given":"L."},{"family":"Nucci","given":"M."},{"family":"Melchers","given":"W.J.G."},{"family":"Verweij","given":"P.E."}],"issued":{"date-parts":[["2015",6]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}]. Resistant *A. fumigatus* strains with TR$_{34}$ and TR$_{46}$ mutations have also been reported among azole-naïve patients in the Middle East, Asia, Africa, Australia, and South America [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"aYpQkszg","properties":{"formattedCitation":"(Chowdhary et al. 2014a, 2017; Meis et al. 2016; Vermeulen et al. 2013; Verweij et al. 2016a)","plainCitation":"(Chowdhary et al. 2014a, 2017;

Meis et al. 2016; Vermeulen et al. 2013; Verweij et al.

2016a)","noteIndex":0},"citationItems":[{"id":244,"uris":["http://zotero.org/users/local/opHgB1Dy/items/RS7AGK55"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/RS7AGK55"],"itemData":{"id":244,"type":"article-journal","abstract":"Azole resistance in Aspergillus fumigatus has emerged as a global health problem. Although the number of cases of azole-resistant aspergillosis is still limited, resistance mechanisms continue to emerge, thereby threatening the role of the azole class in the management of diseases caused by Aspergillus. The majority of cases of azole-resistant disease are due to resistant A. fumigatus originating from the environment. Patient management is difficult due to the absence of patient risk factors, delayed diagnosis, and limited treatment options, resulting in poor treatment outcome. International and collaborative efforts are required to understand how resistance develops in the environment to allow effective measures to be implemented aimed at retaining the use of azoles both for food production and human medicine.","container-title":"Clinical Infectious Diseases","DOI":"10.1093/cid/civ885","ISSN":"1058-4838","issue":"3","journalAbbreviation":"Clin Infect Dis","language":"en","page":"362-368","source":"academic.oup.com","title":"Azole Resistance in Aspergillus fumigatus: Can We Retain the Clinical Use of Mold-Active Antifungal Azoles?","title-short":"Azole Resistance in Aspergillus fumigatus","volume":"62","author":[{"family":"Verweij","given":"Paul E."},{"family":"Chowdhary","given":"Anuradha"},{"family":"Melchers","given":"Willem J. G."},{"family":"Meis","given":"Jacques F."}],"issued":{"date-parts":[["2016",2,1]]}}},{"id":2225,"uris":["http://zotero.org/users/local/opHgB1Dy/items/8FH6MDU4"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/8FH6MDU4"],"itemData":{"id":2225,"type":"article-journal","abstract":": Aspergillus fumigatus, a ubiquitously distributed opportunistic pathogen, is the global leading cause of aspergillosis. Azole antifungals play an important role in the management of aspergillosis. However, over a decade, azole resistance in A. fumigatus isolates has been increasingly

reported with variable prevalence worldwide and it is challenging the effective management of aspergillosis. The high mortality rates observed in patients with invasive aspergillosis caused by azole-resistant A. fumigatus (ARAF) isolates pose serious challenges to the clinical microbiologist for timely identification of resistance and appropriate therapeutic interventions. The majority of ARAF isolates contain alterations in the cyp51A gene; however, there have been increasing reports on non-cyp51A mutations contributing to azole resistant phenotypes. This review highlights the emergence and various mechanisms implicated in the development of azole resistance in A. fumigatus. We further present recent developments related to the environmental route in the emergence of ARAF isolates and discuss the therapeutic options available.","container-title":"Future
Microbiology","DOI":"10.2217/fmb.14.27","ISSN":"1746-
0913","issue":"5","journalAbbreviation":"Future Microbiology","page":"697-
711","source":"futuremedicine.com (Atypon)","title":"Exploring azole antifungal drug resistance in Aspergillus fumigatus with special reference to resistance
mechanisms","volume":"9","author":[{"family":"Chowdhary","given":"Anuradha"},{"family":"Sharma","given":"Cheshta"},{"family":"Hagen","given":"Ferry"},{"family":"Meis","given":"Jacques
F"}],"issued":{"date-
parts":[["2014",5,1]]}}},{"id":2487,"uris":["http://zotero.org/users/local/opHgB1Dy/items/2DZ86BLZ"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/2DZ86BLZ"],"itemData":{"id":2487,"type":"article-journal","abstract":"PURPOSE OF REVIEW: Reports from the end of the 2000s forced the medical community to take azole resistance in Aspergillus fumigatus into account. Not only patients with chronic aspergillus disease, who develop resistance during long-term azole treatment, but also azole-naive patients are at risk, owing to the presence of resistant strains in the environment. The purpose of this review is to overview the latest findings concerning the origin, evolution, and implications of azole resistance in A. fumigatus.\nRECENT FINDINGS: TR34/L98H is the predominant resistance mechanism of

environmental origin in A. fumigatus. Recent epidemiological data show that this mechanism is an expanding problem, with reports from China, Iran, and India. However, the TR34/L98H strains from the Middle East are genotypically different from the European isolates; their emergence is, therefore, not due to simple geographical spread of the 'European' isolates. A new environmental resistance mechanism, TR46/Y121F/T289A, was detected in the Netherlands, conferring voriconazole resistance. In patients chronically treated with triazoles, the spectrum of resistance has become more diverse, with the emergence of non-CYP51A-mediated mechanisms. Central registration of treatment and outcome data of patients with resistant aspergillus disease are needed.\nSUMMARY: Azole resistance in A. fumigatus is evolving to a global health problem.","container-title":"Current Opinion in Infectious Diseases","DOI":"10.1097/QCO.0000000000000005","ISSN":"1473-6527","issue":"6","journalAbbreviation":"Curr. Opin. Infect. Dis.","language":"eng","note":"PMID: 24126719","page":"493-500","source":"PubMed","title":"Azole resistance in Aspergillus fumigatus: a growing public health concern","title-short":"Azole resistance in Aspergillus fumigatus","volume":"26","author":[{"family":"Vermeulen","given":"Edith"},{"family":"Lagrou","given":"Katrien"},{"family":"Verweij","given":"Paul E."}],"issued":{"date-parts":[["2013",12]]}},{"id":2484,"uris":["http://zotero.org/users/local/opHgB1Dy/items/UZ8VCGCH"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/UZ8VCGCH"],"itemData":{"id":2484,"type":"article-journal","abstract":"Abstract. Aspergillus fumigatus remains the most common species in all pulmonary syndromes, followed by Aspergillus flavus which is a common cause of allergic","container-title":"The Journal of Infectious Diseases","DOI":"10.1093/infdis/jix210","ISSN":"0022-1899","issue":"suppl_3","journalAbbreviation":"J Infect Dis","language":"en","page":"S436-S444","source":"academic.oup.com","title":"Azole-Resistant Aspergillosis: Epidemiology, Molecular Mechanisms, and Treatment","title-short":"Azole-Resistant Aspergillosis","volume":"216","author":[{"family":"Chowdhary","given":"Anuradha"},{"family":"Sharma"

,"given":"Cheshta"},{"family":"Meis","given":"Jacques F."}],"issued":{"date-parts":[["2017",8,15]]}}},{"id":2188,"uris":["http://zotero.org/users/local/opHgB1Dy/items/A4AJR7B5"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/A4AJR7B5"],"itemData":{"id":2188,"type":"article-journal","abstract":"Aspergillus fungi are the cause of an array of diseases affecting humans, animals and plants. The triazole antifungal agents itraconazole, voriconazole, isavuconazole and posaconazole are treatment options against diseases caused by Aspergillus. However, resistance to azoles has recently emerged as a new therapeutic challenge in six continents. Although de novo azole resistance occurs occasionally in patients during azole therapy, the main burden is the aquisition of resistance through the environment. In this setting, the evolution of resistance is attributed to the widespread use of azole-based fungicides. Although ubiquitously distributed, A. fumigatus is not a phytopathogen. However, agricultural fungicides deployed against plant pathogenic moulds such as Fusarium, Mycospaerella and A. flavus also show activity against A. fumigatus in the environment and exposure of non-target fungi is inevitable. Further, similarity in molecule structure between azole fungicides and antifungal drugs results in cross-resistance of A. fumigatus to medical azoles. Clinical studies have shown that two-thirds of patients with azole-resistant infections had no previous history of azole therapy and high mortality rates between 50% and 100% are reported in azole-resistant invasive aspergillosis. The resistance phenotype is associated with key mutations in the cyp51A gene, including TR34/L98H, TR53 and TR46/Y121F/T289A resistance mechanisms. Early detection of resistance is of paramount importance and if demonstrated, either with susceptibility testing or through molecular analysis, azole monotherapy should be avoided. Liposomal amphotericin B or a combination of voriconazole and an echinocandin are recomended for azole-resistant aspergillosis.This article is part of the themed issue ‘Tackling emerging fungal threats to animal health, food security and ecosystem resilience’.","container-title":"Philosophical Transactions of the Royal Society B: Biological Sciences","DOI":"10.1098/rstb.2015.0460","issue":"1709","journalAbbreviation":"Philosophical

Transactions of the Royal Society B: Biological Sciences","page":"20150460","source":"royalsocietypublishing.org (Atypon)","title":"Clinical implications of globally emerging azole resistance in Aspergillus fumigatus","volume":"371","author":[{"family":"Meis","given":"Jacques F."},{"family":"Chowdhary","given":"Anuradha"},{"family":"Rhodes","given":"Johanna L."},{"family":"Fisher","given":"Matthew C."},{"family":"Verweij","given":"Paul E."}],"issued":{"date-parts":[["2016",12,5]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}]. Resistant isolates with TR_{34} and TR_{46} mutations have also been detected in environmental samples from Europe, Asia, South America, and East Africa [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"LImgvL8c","properties":{"formattedCitation":"(Alvarez-Moreno et al. 2019; Badali et al. 2013; Chowdhary et al. 2012, 2014b; Dunne et al. 2017; Le Pape et al. 2016; Mortensen et al. 2010; Schoustra et al. 2019; Vermeulen et al. 2012)","plainCitation":"(Alvarez-Moreno et al. 2019; Badali et al. 2013; Chowdhary et al. 2012, 2014b; Dunne et al. 2017; Le Pape et al. 2016; Mortensen et al. 2010; Schoustra et al. 2019; Vermeulen et al. 2012)","noteIndex":0},"citationItems":[{"id":2794,"uris":["http://zotero.org/users/local/opHgB1Dy/items/R55AQP92"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/R55AQP92"],"itemData":{"id":2794,"type":"article-journal","abstract":"A new azole resistance mechanism in Aspergillus fumigatus consisting of a TR46/Y121F/T289A alteration in the cyp51A gene was recently described in the Netherlands. Strains containing these mutations are associated with invasive infection and therapy failure. This communication describes the first case of fatal invasive aspergillosis caused by TR46/Y121F/T289A outside the Netherlands, in the neighboring country of Belgium, suggesting geographical spread. TR46/Y121F/T289A leads to a recognisable phenotypic susceptibility pattern which should trigger cyp51A genotyping to monitor further spread.","container-title":"Eurosurveillance","DOI":"10.2807/ese.17.48.20326-en","ISSN":"1560-

7917","issue":"48","language":"en","page":"20326","source":"www.eurosurveillance.org","title":"Azole-resistant Aspergillus fumigatus due to TR46/Y121F/T289A mutation emerging in Belgium, July 2012","volume":"17","author":[{"family":"Vermeulen","given":"E."},{"family":"Maertens","given":"J."},{"family":"Schoemans","given":"H."},{"family":"Lagrou","given":"K."}],"issued":{"date-parts":[["2012",11,29]]}}},{"id":2806,"uris":["http://zotero.org/users/local/opHgB1Dy/items/ATYYTHBB"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/ATYYTHBB"],"itemData":{"id":2806,"type":"article-journal","abstract":"AbstractObjectives. Azole resistance in Aspergillus fumigatus isolates has been increasingly reported with variable prevalence worldwide and is challenging the","container-title":"Journal of Antimicrobial Chemotherapy","DOI":"10.1093/jac/dku259","ISSN":"0305-7453","issue":"11","journalAbbreviation":"J Antimicrob Chemother","language":"en","page":"2979-2983","source":"academic.oup.com","title":"Multi-azole-resistant Aspergillus fumigatus in the environment in Tanzania","volume":"69","author":[{"family":"Chowdhary","given":"Anuradha"},{"family":"Sharma","given":"Cheshta"},{"family":"Boom","given":"Mara","non-dropping-particle":"van den"},{"family":"Yntema","given":"Jan Bart"},{"family":"Hagen","given":"Ferry"},{"family":"Verweij","given":"Paul E."},{"family":"Meis","given":"Jacques F."}],"issued":{"date-parts":[["2014",11,1]]}}},{"id":2476,"uris":["http://zotero.org/users/local/opHgB1Dy/items/R8M6S9I6"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/R8M6S9I6"],"itemData":{"id":2476,"type":"article-journal","container-title":"Emerging Infectious Diseases","DOI":"10.3201/eid2201.150978","ISSN":"1080-6040","issue":"1","journalAbbreviation":"Emerg Infect Dis","note":"PMID: 26690795\nPMCID: PMC4696698","page":"156-157","source":"PubMed Central","title":"Multiple Fungicide-Driven Alterations in Azole-Resistant Aspergillus fumigatus, Colombia,

2015","volume":"22","author":[{"family":"Le
Pape","given":"Patrice"},{"family":"Lavergne","given":"Rose-
Anne"},{"family":"Morio","given":"Florent"},{"family":"Alvarez-
Moreno","given":"Carlos"}],"issued":{"date-
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ri":["http://zotero.org/users/local/opHgB1Dy/items/P64GFWZV"],"itemData":{"id":3072,"type":"article-
journal","abstract":"Aspergillus fumigatus resistant to azole as first-line therapy has been reported in azole-naïve patients. This worldwide resistance phenomenon has been linked to fungicide-driven alterations in the cyp51A gene and its promoter region (such as TR34/L98H and TR46/Y121F/T289A). Azole-resistant A. fumigatus related to the use of triazole fungicides in flower fields was recently reported In Colombia. The purpose of this study was to investigate the presence of azole-resistant A. fumigatus in soil samples from vegetable crops such as carrots, potatoes, maize, strawberries, and pea, and from prepared farming land surrounding the city of Bogotá. Species identification was based on sequencing of the β-tubulin and calmodulin genes. All A. fumigatus strains were screened for azole resistance on agar supplemented with itraconazole or voriconazole. Among the 60 soil samples, 34 (56.6%) were positive for A. fumigatus and 15 samples exhibited strains (n = 18) that grew on agar supplemented with itraconazole or voriconazole. Triazoleresistant strains were isolated from soil samples associated with carrot, potato, maize, and pea crops. Sequencing of the cyp51A gene and its promoter region indicated polymorphism, mainly with the presence of TR46/Y121F/T289A (n = 8), TR34/L98H, and TR53. Eight resistant isolates exhibited cyp51A wild type without alterations in the promoter region. Our study showed evidence of dissemination of azoleresistant A. fumigatus, with high genetic diversity, in vegetable crops in Colombia. These data underline the need to determine the prevalence of azole resistance in A. fumigatus in clinical and environmental settings for other regions of Colombia as well as Latin America.","container-

title":"Mycologia","DOI":"10.1080/00275514.2018.1557796","ISSN":"0027-5514, 1557-2536","issue":"2","journalAbbreviation":"Mycologia","language":"en","page":"217-224","source":"DOI.org (Crossref)","title":"Fungicide-driven alterations in azole-resistant <i>Aspergillus fumigatus</i> are related to vegetable crops in Colombia, South America","volume":"111","author":[{"family":"Alvarez-Moreno","given":"Carlos"},{"family":"Lavergne","given":"Rose-Anne"},{"family":"Hagen","given":"Ferry"},{"family":"Morio","given":"Florent"},{"family":"Meis","given":"Jacques F."},{"family":"Le Pape","given":"Patrice"}],"issued":{"date-parts":[["2019",3,4]]}}},{"id":2671,"uris":["http://zotero.org/users/local/opHgB1Dy/items/9Q6YBPE8"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/9Q6YBPE8"],"itemData":{"id":2671,"type":"article-journal","abstract":"A single mechanism of azole resistance was shown to predominate in clinical and environmental Aspergillus fumigatus isolates from the Netherlands, and a link to the use of azoles in the environment was suggested. To explore the prevalence of azole-resistant A. fumigatus and other aspergilli in the environment in other European countries, we collected samples from the surroundings of hospitals in Copenhagen, Innsbruck, and Madrid, flowerbeds in an amusement park in Copenhagen, and compost bags purchased in Austria, Denmark, and Spain and screened for azole resistance using multidish agars with itraconazole, voriconazole, and posaconazole. EUCAST method E.DEF 9.1 was used to confirm azole resistance. The promoter and entire coding sequence of the cyp51A gene were sequenced to identify azole-resistant A. fumigatus isolates. A. fumigatus was recovered in 144 out of 185 samples (77.8%). Four A. fumigatus isolates from four Danish soil samples displayed elevated azole MICs (8%), and all harbored the same TR/L98H mutation of cyp51A. One A. lentulus isolate with voriconazole MIC of 4 mg/liter was detected in Spain. No azole-resistant aspergilli were detected in compost. Finally, A. terreus was present in seven samples from Austria. Multi-azole-resistant A. fumigatus is present in the environment in Denmark. The resistance mechanism is identical to that of

environmental isolates in the Netherlands. No link to commercial compost could be detected. In Spain
and Austria, only Aspergillus species with intrinsic resistance to either azoles or amphotericin B were
found.","container-title":"Antimicrobial Agents and Chemotherapy","DOI":"10.1128/AAC.00692-
10","ISSN":"0066-4804, 1098-6596","issue":"11","language":"en","note":"PMID:
20805399","page":"4545-4549","source":"aac.asm.org","title":"Environmental Study of Azole-Resistant
Aspergillus fumigatus and Other Aspergilli in Austria, Denmark, and
Spain","volume":"54","author":[{"family":"Mortensen","given":"Klaus
Leth"},{"family":"Mellado","given":"Emilia"},{"family":"Lass-
Flörl","given":"Cornelia"},{"family":"Rodriguez-Tudela","given":"Juan
Luis"},{"family":"Johansen","given":"Helle Krogh"},{"family":"Arendrup","given":"Maiken
Cavling"}],"issued":{"date-
parts":[["2010",11,1]]}}},{"id":3202,"uris":["http://zotero.org/users/local/opHgB1Dy/items/33ARLVJN"],
"uri":["http://zotero.org/users/local/opHgB1Dy/items/33ARLVJN"],"itemData":{"id":3202,"type":"article
-journal","abstract":"Azole resistance is an emerging problem in Aspergillus which impacts the
management of aspergillosis. Here in we report the emergence and clonal spread of resistance to
triazoles in environmental Aspergillus fumigatus isolates in India. A total of 44 (7%) A. fumigatus isolates
from 24 environmental samples were found to be triazole resistant. The isolation rate of resistant A.
fumigatus was highest (33%) from soil of tea gardens followed by soil from flower pots of the hospital
garden (20%), soil beneath cotton trees (20%), rice paddy fields (12.3%), air samples of hospital wards
(7.6%) and from soil admixed with bird droppings (3.8%). These strains showed cross-resistance to
voriconazole, posaconazole, itraconazole and to six triazole fungicides used extensively in agriculture.
Our analyses identified that all triazole-resistant strains from India shared the same TR34/L98H mutation
in the cyp51 gene. In contrast to the genetic uniformity of azole-resistant strains the azole-susceptible
isolates from patients and environments in India were genetically very diverse. All nine loci were highly

polymorphic in populations of azole-susceptible isolates from both clinical and environmental samples. Furthermore, all Indian environmental and clinical azole resistant isolates shared the same multilocus microsatellite genotype not found in any other analyzed samples, either from within India or from the Netherlands, France, Germany or China. Our population genetic analyses suggest that the Indian azole-resistant A. fumigatus genotype was likely an extremely adaptive recombinant progeny derived from a cross between an azole-resistant strain migrated from outside of India and a native azole-susceptible strain from within India, followed by mutation and then rapid dispersal through many parts of India. Our results are consistent with the hypothesis that exposure of A. fumigatus to azole fungicides in the environment causes cross-resistance to medical triazoles. The study emphasises the need of continued surveillance of resistance in environmental and clinical A. fumigatus strains.","container-title":"PLOS ONE","DOI":"10.1371/journal.pone.0052871","ISSN":"1932-6203","issue":"12","journalAbbreviation":"PLOS ONE","language":"en","page":"e52871","source":"PLoS Journals","title":"Clonal Expansion and Emergence of Environmental Multiple-Triazole-Resistant Aspergillus fumigatus Strains Carrying the TR34/L98H Mutations in the cyp51A Gene in India","volume":"7","author":[{"family":"Chowdhary","given":"Anuradha"},{"family":"Kathuria","given":"Shallu"},{"family":"Xu","given":"Jianping"},{"family":"Sharma","given":"Cheshta"},{"family":"Sundar","given":"Gandhi"},{"family":"Singh","given":"Pradeep Kumar"},{"family":"Gaur","given":"Shailendra N."},{"family":"Hagen","given":"Ferry"},{"family":"Klaassen","given":"Corné H."},{"family":"Meis","given":"Jacques F."}],"issued":{"date-parts":[["2012",12,28]]}}},{"id":3205,"uris":["http://zotero.org/users/local/opHgB1Dy/items/WJ24BAZU"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/WJ24BAZU"],"itemData":{"id":3205,"type":"article-journal","abstract":"Azole resistance in Aspergillus is emerging in European and Asian countries. As azoles are mainstay of therapy in the management of aspergillosis, azole resistance has serious implications in patient management. We report the emergence of resistance to triazoles in environmental Aspergillus fumigatus isolates in Iran. The TR34/L98H mutation was the only resistance mechanism. Overall 3.3% of the A. fumigatus isolates from hospital surroundings in Sari and Tehran had the same TR34/L98H STRAf genotype and were related to some resistant clinical and environmental TR34/L98H isolates from the Netherlands and India. It is emphasised that routine resistance surveillance studies focusing on environmental and clinical samples are warranted to yield the true prevalence of azole resistance in A. fumigatus in Iran.","container-title":"Mycoses","DOI":"10.1111/myc.12089","ISSN":"1439-0507","issue":"6","language":"en","page":"659-663","source":"Wiley Online Library","title":"Environmental study of azole-resistant Aspergillus fumigatus with TR34/L98H mutations in the cyp51A gene in Iran","volume":"56","author":[{"family":"Badali","given":"Hamid"},{"family":"Vaezi","given":"Afsane"},{"family":"Haghani","given":"Iman"},{"family":"Yazdanparast","given":"Seyed A."},{"family":"Hedayati","given":"Mohammad T."},{"family":"Mousavi","given":"Bita"},{"family":"Ansari","given":"Saham"},{"family":"Hagen","given":"Ferry"},{"family":"Meis","given":"Jacques F."},{"family":"Chowdhary","given":"Anuradha"}],"issued":{"date-parts":[["2013"]]}}},{"id":2247,"uris":["http://zotero.org/users/local/opHgB1Dy/items/DJIYXWPP"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/DJIYXWPP"],"itemData":{"id":2247,"type":"article-journal","abstract":"Azole resistance is a major concern for treatment of infections with Aspergillus fumigatus. Environmental resistance selection is a main route for Aspergillus spp. to acquire azole resistance. We investigated the presence of environmental hotspots for resistance selection in the Netherlands on the basis of the ability of A. fumigatus to grow and reproduce in the presence of azole fungicide residues. We identified 3 hotspots: flower bulb waste, green waste material, and wood chippings. We recovered azole-resistant A. fumigatus from these sites; all fungi contained cyp51A tandem repeat–mediated resistance mechanisms identical to those found in clinical isolates. Tebuconazole, epoxiconazole, and prothioconazole were the most frequently found fungicide residues. Stockpiles of plant waste contained the highest levels of azole-resistant A. fumigatus, and active aerobic composting reduced Aspergillus colony counts. Preventing plant waste stockpiling or creating unfavorable conditions for A. fumigatus to grow in stockpiles might reduce environmental resistance burden.","container-title":"Emerging Infectious Diseases","DOI":"10.3201/eid2507.181625","ISSN":"1080-6040","issue":"7","journalAbbreviation":"Emerg Infect Dis","note":"PMID: 31211684\nPMCID: PMC6590754","page":"1347-1353","source":"PubMed Central","title":"Environmental Hotspots for Azole Resistance Selection of Aspergillus fumigatus, the Netherlands","volume":"25","author":[{"family":"Schoustra","given":"Sijmen E."},{"family":"Debets","given":"Alfons J.M."},{"family":"Rijs","given":"Antonius J.M.M."},{"family":"Zhang","given":"Jianhua"},{"family":"Snelders","given":"Eveline"},{"family":"Leendertse","given":"Peter C."},{"family":"Melchers","given":"Willem J.G."},{"family":"Rietveld","given":"Anton G."},{"family":"Zwaan","given":"Bas J."},{"family":"Verweij","given":"Paul E."}],"issued":{"date-parts":[["2019",7]]}}},{"id":2456,"uris":["http://zotero.org/users/local/opHgB1Dy/items/G5R9NHR8"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/G5R9NHR8"],"itemData":{"id":2456,"type":"article-journal","container-title":"Clinical Infectious Diseases","DOI":"10.1093/cid/cix257","ISSN":"1058-4838, 1537-6591","issue":"1","language":"en","page":"147-149","source":"DOI.org (Crossref)","title":"Intercountry Transfer of Triazole-Resistant Aspergillus fumigatus on Plant Bulbs","volume":"65","author":[{"family":"Dunne","given":"Katie"},{"family":"Hagen","given":"Ferry"},{"family":"Pomeroy","given":"Niamh"},{"family":"Meis","given":"Jacques F"},{"family":"Rogers","given":"Thomas R"}],"issued":{"date-parts":[["2017",7,1]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}]. Further supporting a link between fungicide use and clinical resistance, triazole fungicides similar to medical antifungals were introduced for agricultural use in the Netherlands just before the first TR$_{34}$ strain was found in human clinical settings in the late-1990s [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"xtTZYhxV","properties":{"formattedCitation":"(Meis et al. 2016)","plainCitation":"(Meis et al. 2016)","noteIndex":0},"citationItems":[{"id":2188,"uris":["http://zotero.org/users/local/opHgB1Dy/items/A4AJR7B5"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/A4AJR7B5"],"itemData":{"id":2188,"type":"article-journal","abstract":"Aspergillus fungi are the cause of an array of diseases affecting humans, animals and plants. The triazole antifungal agents itraconazole, voriconazole, isavuconazole and posaconazole are treatment options against diseases caused by Aspergillus. However, resistance to azoles has recently emerged as a new therapeutic challenge in six continents. Although de novo azole resistance occurs occasionally in patients during azole therapy, the main burden is the aquisition of resistance through the environment. In this setting, the evolution of resistance is attributed to the widespread use of azole-based fungicides. Although ubiquitously distributed, A. fumigatus is not a phytopathogen. However, agricultural fungicides deployed against plant pathogenic moulds such as Fusarium, Mycospaerella and A. flavus also show activity against A. fumigatus in the environment and exposure of non-target fungi is inevitable. Further, similarity in molecule structure between azole fungicides and antifungal drugs results in cross-resistance of A. fumigatus to medical azoles. Clinical studies have shown that two-thirds of patients with azole-resistant infections had no previous history of azole therapy and high mortality rates between 50% and 100% are reported in azole-resistant invasive aspergillosis. The resistance phenotype is associated with key mutations in the cyp51A gene, including TR34/L98H, TR53 and TR46/Y121F/T289A resistance mechanisms. Early detection of resistance is of paramount importance and if demonstrated, either with susceptibility testing or through molecular analysis, azole monotherapy should be avoided. Liposomal amphotericin B or a combination of voriconazole and an echinocandin are recomended for azole-resistant aspergillosis.This article is part of the themed issue 'Tackling emerging fungal threats to animal health, food security and ecosystem resilience'.","container-title":"Philosophical Transactions of the Royal Society B: Biological Sciences","DOI":"10.1098/rstb.2015.0460","issue":"1709","journalAbbreviation":"Philosophical Transactions of the Royal Society B: Biological Sciences","page":"20150460","source":"royalsocietypublishing.org (Atypon)","title":"Clinical implications of globally emerging azole resistance in Aspergillus fumigatus","volume":"371","author":[{"family":"Meis","given":"Jacques F."},{"family":"Chowdhary","given":"Anuradha"},{"family":"Rhodes","given":"Johanna L."},{"family":"Fisher","given":"Matthew C."},{"family":"Verweij","given":"Paul E."}],"issued":{"date-parts":[["2016",12,5]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}].

In the United States, a small number of infections caused by resistant *A. fumigatus* strains have been identified [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"GjiTTYiV","properties":{"formattedCitation":"(CDC 2019)","plainCitation":"(CDC 2019)","noteIndex":0},"citationItems":[{"id":3108,"uris":["http://zotero.org/users/local/opHgB1Dy/items/CY54CY6Z"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/CY54CY6Z"],"itemData":{"id":3108,"type":"report","event-place":"Atlanta, GA","language":"en","page":"148","publisher":"U.S. Department of Health and Human Services, CDC","publisher-place":"Atlanta, GA","source":"Zotero","title":"Antibiotic Resistance Threats in the United States, 2019","URL":"https://www.cdc.gov/drugresistance/biggest-threats.html","author":[{"literal":"CDC"}],"issued":{"date-parts":[["2019"]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}]. The first TR-based resistance in patients was reported in 2016, including retrospectively identified isolates (two TR_{34} and two TR_{46}) collected as early as 2008 [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"3r2FG7Oz","properties":{"formattedCitation":"(Vazquez and Manavathu 2016; Wiederhold et al. 2016)","plainCitation":"(Vazquez and Manavathu 2016; Wiederhold et al. 2016)","noteIndex":0},"citationItems":[{"id":2490,"uris":["http://zotero.org/users/local/opHgB1Dy/items/88D4S5BY"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/88D4S5BY"],"itemData":{"id":2490,"type":"article-journal","abstract":"Azole resistance in Aspergillus fumigatus is an increasing problem. The TR34 L98H and TR46 Y121F T289A mutations that can occur in patients without previous azole exposure have been reported in Europe, Asia, the Middle East, Africa, and Australia. Here, we report the detection of both the TR34 L98H and TR46 Y121F T289A mutations in confirmed A. fumigatus isolates collected in institutions in the United States. These mutations, other mutations known to cause azole resistance, and azole MICs are reported here.","container-title":"Journal of Clinical Microbiology","DOI":"10.1128/JCM.02478-15","ISSN":"0095-1137, 1098-660X","issue":"1","language":"en","note":"PMID: 26491179","page":"168-171","source":"jcm.asm.org","title":"First Detection of TR34 L98H and TR46 Y121F T289A Cyp51 Mutations in Aspergillus fumigatus Isolates in the United States","volume":"54","author":[{"family":"Wiederhold","given":"Nathan P."},{"family":"Gil","given":"Veronica Garcia"},{"family":"Gutierrez","given":"Felipe"},{"family":"Lindner","given":"Jonathan R."},{"family":"Albataineh","given":"Mohammad T."},{"family":"McCarthy","given":"Dora I."},{"family":"Sanders","given":"Carmita"},{"family":"Fan","given":"Hongxin"},{"family":"Fothergill","given":"Annette W."},{"family":"Sutton","given":"Deanna A."}],"issued":{"date-parts":[["2016",1,1]]}}},{"id":3199,"uris":["http://zotero.org/users/local/opHgB1Dy/items/XQR92NFP"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/XQR92NFP"],"itemData":{"id":3199,"type":"article

-journal","abstract":"Molecular characterization of cyp51A from the azole-resistant Aspergillus fumigatus isolate 50593 from a lung transplant patient showed Y121F/T289A changes coupled with a 46-bp tandem repeat (TR46) on the promoter, whereas cyp51A from the pretherapy isolate, A. fumigatus 47381, showed no changes. This is the first reported case of A. fumigatus azole resistance due to Y121F/T289A/TR46 in the United States, suggesting that multiple mutational alterations of cyp51A resulting in high-level azole resistance could occur during prolonged antifungal therapy.","container-title":"Antimicrobial Agents and Chemotherapy","DOI":"10.1128/AAC.01130-15","ISSN":"0066-4804","issue":"2","journalAbbreviation":"Antimicrob Agents Chemother","note":"PMID: 26574014\nPMCID: PMC4750678","page":"1129-1133","source":"PubMed Central","title":"Molecular Characterization of a Voriconazole-Resistant, Posaconazole-Susceptible Aspergillus fumigatus Isolate in a Lung Transplant Recipient in the United States","volume":"60","author":[{"family":"Vazquez","given":"Jose A."},{"family":"Manavathu","given":"Elias K."}],"issued":{"date-parts":[["2016",1,29]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}]. An additional 6 isolates were detected through 2018 [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"p3lYp8n7","properties":{"formattedCitation":"(Beer 2018)","plainCitation":"(Beer 2018)","noteIndex":0},"citationItems":[{"id":2169,"uris":["http://zotero.org/users/local/opHgB1Dy/items/J83LUV2C"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/J83LUV2C"],"itemData":{"id":2169,"type":"article-journal","abstract":"The environmental mold Aspergillus fumigatus is the primary cause of invasive aspergillosis. In patients with high-risk conditions, including stem cell and organ transplant recipients, mortality ...","container-title":"MMWR. Morbidity and Mortality Weekly Report","DOI":"10.15585/mmwr.mm6738a5","ISSN":"0149-21951545-861X","journalAbbreviation":"MMWR Morb Mortal Wkly Rep","language":"en-us","source":"www.cdc.gov","title":"Multidrug-Resistant Aspergillus fumigatus Carrying Mutations Linked to Environmental Fungicide Exposure — Three States, 2010–2017","URL":"https://www.cdc.gov/mmwr/volumes/67/wr/mm6738a5.htm","volume":"67","author":[{"family":"Beer","given":"Karlyn D."}],"accessed":{"date-parts":[["2019",7,5]]},"issued":{"date-parts":[["2018"]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}]. Together, these 10 isolates likely reflect only a small proportion of the true burden given lack of standardized surveillance and limited clinical testing. Resistant *A. fumigatus* strains with the TR_{34} mutation have also been found in peanut crop debris in the U.S. state of Georgia that had been treated with propiconazole and tebuconazole, triazoles that are structurally similar to medical triazoles [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"sN7fb8UC","properties":{"formattedCitation":"(Hurst et al. 2017)","plainCitation":"(Hurst et al. 2017)","noteIndex":0},"citationItems":[{"id":250,"uris":["http://zotero.org/users/local/opHgB1Dy/items/FH424KED"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/FH424KED"],"itemData":{"id":250,"type":"article-journal","abstract":"BackgroundAzole resistance in isolates of the fungus Aspergillus fumigatus has been associated with agricultural use of azole fungicides. Environmental isolation of resistant isolates has been reported in Asia, Africa, Europe and South America.ObjectivesTo determine whether A. fumigatus isolates containing TR34/L98H or TR46/Y121F/T289A can be found in fields in the USA treated with agricultural azoles.MethodsCrop debris was collected and screened for A. fumigatus. All A. fumigatus isolates were screened for azole resistance. The CYP51A gene of azole-resistant isolates was sequenced. The population structure of a subset of isolates was determined using microsatellite typing.ResultsThis article identifies azole-resistant A. fumigatus isolates containing the TR34/L98H mutation in an experimental peanut field that had been treated with azole fungicides.ConclusionsThese findings suggest the development of resistance to azole antifungals in A. fumigatus may be present where agricultural azoles are used in the USA.","container-title":"Journal of Antimicrobial Chemotherapy","DOI":"10.1093/jac/dkx168","ISSN":"0305-7453","issue":"9","journalAbbreviation":"J Antimicrob Chemother","language":"en","page":"2443-2446","source":"academic.oup.com","title":"Isolation of azole-resistant Aspergillus fumigatus from the environment in the south-eastern USA","volume":"72","author":[{"family":"Hurst","given":"Steven F."},{"family":"Berkow","given":"Elizabeth L."},{"family":"Stevenson","given":"Katherine L."},{"family":"Litvintseva","given":"Anastasia P."},{"family":"Lockhart","given":"Shawn R."}],"issued":{"date-parts":[["2017",9,1]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}], demonstrating this resistance was also present in the U.S. agricultural environment. Because of this emergence in the United States, CDC has placed triazole-resistant *A. fumigatus* on its “Watch List” for antimicrobial resistance threats [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"k5oDnj5T","properties":{"formattedCitation":"(CDC 2019)","plainCitation":"(CDC 2019)","noteIndex":0},"citationItems":[{"id":3108,"uris":["http://zotero.org/users/local/opHgB1Dy/items/CY54CY6Z"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/CY54CY6Z"],"itemData":{"id":3108,"type":"report","event-place":"Atlanta, GA","language":"en","page":"148","publisher":"U.S. Department of Health and Human Services, CDC","publisher-place":"Atlanta, GA","source":"Zotero","title":"Antibiotic Resistance Threats in the United States, 2019","URL":"https://www.cdc.gov/drugresistance/biggest-threats.html","author":[{"literal":"CDC"}],"issued":{"date-parts":[["2019"]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}].

Given increased global incidence of triazole-resistant *Aspergillus* infections, recent identification of triazole resistance mechanisms linked to agricultural fungicide use in the United States, and triazole agricultural fungicides with the same mechanism of action as triazole antifungal medications, we characterized trends in U.S. agricultural triazole use to explore possible implications for antifungal resistant human infections.

Methods

We analyzed publicly available estimates of annual agricultural pesticide use from the U.S. Geological Survey (USGS) [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"ngeYst6S","properties":{"formattedCitation":"(Baker and Stone 2015; Stone 2013; Thelin and Stone 2013)","plainCitation":"(Baker and Stone 2015; Stone 2013; Thelin and Stone 2013)","noteIndex":0},"citationItems":[{"id":2414,"uris":["http://zotero.org/users/local/opHgB1Dy/items/UTJNBZWL"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/UTJNBZWL"],"itemData":{"id":2414,"type":"report","title":"Estimation of Annual Agricultural Pesticide Use for Counties of the Conterminous United States, 1992–2009","URL":"https://pubs.usgs.gov/sir/2013/5009/","author":[{"family":"Thelin","given":"Gail P"},{"family":"Stone","given":"Wesley W"}],"accessed":{"date-parts":[["2019",7,20]]},"issued":{"date-parts":[["2013"]]}}},{"id":2416,"uris":["http://zotero.org/users/local/opHgB1Dy/items/CKJ6QWHE"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/CKJ6QWHE"],"itemData":{"id":2416,"type":"report","title":"Estimated Annual Agricultural Pesticide Use for Counties of the Conterminous United States, 1992–2009","URL":"https://pubs.usgs.gov/ds/752/","author":[{"family":"Stone","given":"Wesley W"}],"accessed":{"date-parts":[["2019",7,20]]},"issued":{"date-parts":[["2013"]]}}},{"id":2467,"uris":["http://zotero.org/users/local/opHgB1Dy/items/SFVBG7EQ"],"uri"

:["http://zotero.org/users/local/opHgB1Dy/items/SFVBG7EQ"],"itemData":{"id":2467,"type":"report","number":"Data Series 907","title":"Estimated Annual Agricultural Pesticide Use for Counties of the Conterminous United States, 2008–12","URL":"https://pubs.usgs.gov/ds/0907/","author":[{"family":"Baker","given":"Nancy T"},{"family":"Stone","given":"Wesley W"}],"accessed":{"date-parts":[["2019",7,20]]},"issued":{"date-parts":[["2015"]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}] for 16 triazole fungicides used in the United States during 1992–2016 [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"1DS6mgbV","properties":{"formattedCitation":"(USGS 2017)","plainCitation":"(USGS 2017)","noteIndex":0},"citationItems":[{"id":2464,"uris":["http://zotero.org/users/local/opHgB1Dy/items/KB9ZIEZT"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/KB9ZIEZT"],"itemData":{"id":2464,"type":"webpage","title":"USGS NAWQA: The Pesticide National Synthesis Project","URL":"https://water.usgs.gov/nawqa/pnsp/usage/maps/about.php","author":[{"literal":"USGS"}],"accessed":{"date-parts":[["2019",7,20]]},"issued":{"date-parts":[["2017"]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}]. Data for District of Colombia, Hawaii, Alaska, and territories were not included in the estimates. Methods for these estimates are described in detail elsewhere [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"70RoxqRB","properties":{"formattedCitation":"(Baker and Stone 2015; Stone 2013; Thelin and Stone 2013)","plainCitation":"(Baker and Stone 2015; Stone 2013; Thelin and Stone 2013)","noteIndex":0},"citationItems":[{"id":2414,"uris":["http://zotero.org/users/local/opHgB1Dy/items/UTJNBZWL"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/UTJNBZWL"],"itemData":{"id":2414,"type":"report","title":"Estimation of Annual Agricultural Pesticide Use for Counties of the Conterminous United States, 1992–

2009","URL":"https://pubs.usgs.gov/sir/2013/5009/","author":[{"family":"Thelin","given":"Gail
P"},{"family":"Stone","given":"Wesley W"}],"accessed":{"date-parts":[["2019",7,20]]},"issued":{"date-
parts":[["2013"]]}}},{"id":2416,"uris":["http://zotero.org/users/local/opHgB1Dy/items/CKJ6QWHE"],"uri
":["http://zotero.org/users/local/opHgB1Dy/items/CKJ6QWHE"],"itemData":{"id":2416,"type":"report","
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1992–2009","URL":"https://pubs.usgs.gov/ds/752/","author":[{"family":"Stone","given":"Wesley
W"}],"accessed":{"date-parts":[["2019",7,20]]},"issued":{"date-
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:["http://zotero.org/users/local/opHgB1Dy/items/SFVBG7EQ"],"itemData":{"id":2467,"type":"report","n
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Conterminous United States, 2008–
12","URL":"https://pubs.usgs.gov/ds/0907/","author":[{"family":"Baker","given":"Nancy
T"},{"family":"Stone","given":"Wesley W"}],"accessed":{"date-parts":[["2019",7,20]]},"issued":{"date-
parts":[["2015"]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-
citation.json"}]. Briefly, estimates of pesticide use were compiled by proprietary surveys of farms, California Department of Pesticide Regulation Pesticide Use Reports (DPR-PURs), and the United States Department of Agricultural National Agricultural Statistics Service (NASS) to estimate the use per harvested-crop acreage for each crop by year. Low and high estimates of pesticide use are stratified by pesticide, county, crop type, and year. For years with unavailable data in a given county, low estimates report zero rates and high estimates incorporated rates from nearby areas [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"GZQXH9zF","properties":{"formattedCitation":"(Baker and Stone 2015; Stone 2013; Thelin and Stone 2013)","plainCitation":"(Baker and Stone 2015; Stone 2013; Thelin and Stone
2013)","noteIndex":0},"citationItems":[{"id":2414,"uris":["http://zotero.org/users/local/opHgB1Dy/item

s/UTJNBZWL"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/UTJNBZWL"],"itemData":{"id":2414,"type":"report","title":"Estimation of Annual Agricultural Pesticide Use for Counties of the Conterminous United States, 1992–2009","URL":"https://pubs.usgs.gov/sir/2013/5009/","author":[{"family":"Thelin","given":"Gail P"},{"family":"Stone","given":"Wesley W"}],"accessed":{"date-parts":[["2019",7,20]]},"issued":{"date-parts":[["2013"]]}}},{"id":2416,"uris":["http://zotero.org/users/local/opHgB1Dy/items/CKJ6QWHE"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/CKJ6QWHE"],"itemData":{"id":2416,"type":"report","title":"Estimated Annual Agricultural Pesticide Use for Counties of the Conterminous United States, 1992–2009","URL":"https://pubs.usgs.gov/ds/752/","author":[{"family":"Stone","given":"Wesley W"}],"accessed":{"date-parts":[["2019",7,20]]},"issued":{"date-parts":[["2013"]]}}},{"id":2467,"uris":["http://zotero.org/users/local/opHgB1Dy/items/SFVBG7EQ"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/SFVBG7EQ"],"itemData":{"id":2467,"type":"report","number":"Data Series 907","title":"Estimated Annual Agricultural Pesticide Use for Counties of the Conterminous United States, 2008–12","URL":"https://pubs.usgs.gov/ds/0907/","author":[{"family":"Baker","given":"Nancy T"},{"family":"Stone","given":"Wesley W"}],"accessed":{"date-parts":[["2019",7,20]]},"issued":{"date-parts":[["2015"]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}].

Sixteen triazoles in the USGS dataset are used primarily as fungicides. Because seven of these triazoles (difenoconazole, metconazole, myclobutanil, propiconazole, prothiconazole, tebuconazole, and triadimefon) accounted for 93% of triazole use, we grouped the remaining nine fungicides (cyproconazole, fenbuconazole, flusilazole, flutriafol, ipconazole, tetraconazole, triadimenol, triticonazole, and uniconazole) into a single category. Three of the five agricultural triazoles documented to be similar to medical triazoles [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"tXBoC1xU","properties":{"formattedCitation":"(Snelders et al. 2012)","plainCitation":"(Snelders et al. 2012)","noteIndex":0},"citationItems":[{"id":2272,"uris":["http://zotero.org/users/local/opHgB1Dy/items/XSZI44QZ"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/XSZI44QZ"],"itemData":{"id":2272,"type":"article-journal","abstract":"Background Azoles play an important role in the management of Aspergillus diseases. Azole resistance is an emerging global problem in Aspergillus fumigatus, and may develop through patient therapy. In addition, an environmental route of resistance development has been suggested through exposure to 14α-demethylase inhibitors (DMIs). The main resistance mechanism associated with this putative fungicide-driven route is a combination of alterations in the Cyp51A-gene (TR34/L98H). We investigated if TR34/L98H could have developed through exposure to DMIs. Methods and Findings Thirty-one compounds that have been authorized for use as fungicides, herbicides, herbicide safeners and plant growth regulators in the Netherlands between 1970 and 2005, were investigated for cross-resistance to medical triazoles. Furthermore, CYP51-protein homology modeling and molecule alignment studies were performed to identify similarity in molecule structure and docking modes. Five triazole DMIs, propiconazole, bromuconazole, tebuconazole, epoxiconazole and difenoconazole, showed very similar molecule structures to the medical triazoles and adopted similar poses while docking the protein. These DMIs also showed the greatest cross-resistance and, importantly, were authorized for use between 1990 and 1996, directly preceding the recovery of the first clinical TR34/L98H isolate in 1998. Through microsatellite genotyping of TR34/L98H isolates we were able to calculate that the first isolate would have arisen in 1997, confirming the results of the abovementioned experiments. Finally, we performed induction experiments to investigate if TR34/L98H could be induced under laboratory conditions. One isolate evolved from two copies of the tandem repeat to three, indicating that fungicide pressure can indeed result in these genomic changes.

Conclusions Our findings support a fungicide-driven route of TR34/L98H development in A. fumigatus. Similar molecule structure characteristics of five triazole DMIs and the three medical triazoles appear the underlying mechanism of cross resistance development. Our findings have major implications for the assessment of health risks associated with the use of triazole DMIs.","container-title":"PLOS ONE","DOI":"10.1371/journal.pone.0031801","ISSN":"1932-6203","issue":"3","journalAbbreviation":"PLOS ONE","language":"en","page":"e31801","source":"PLoS Journals","title":"Triazole Fungicides Can Induce Cross-Resistance to Medical Triazoles in Aspergillus fumigatus","volume":"7","author":[{"family":"Snelders","given":"Eveline"},{"family":"Camps","given":"Simone M. T."},{"family":"Karawajczyk","given":"Anna"},{"family":"Schaftenaar","given":"Gijs"},{"family":"Kema","given":"Gert H. J."},{"family":"Lee","given":"Henrich A.","dropping-particle":"van der"},{"family":"Klaassen","given":"Corné H."},{"family":"Melchers","given":"Willem J. G."},{"family":"Verweij","given":"Paul E."}],"issued":{"date-parts":[["2012",3,1]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}] are registered for use in the United States (difenoconazole, propiconazole, and tebuconazole).

We grouped crops into eight categories: corn, cotton, orchards and grapes (stone fruit trees, citrus, nut trees, apples, pears, and grapevines), rice, soybeans, vegetables and fruit (vegetables and non-orchard fruit, including beans, peas, greens, berries, and melons), wheat, and other crops. The other crop category includes pasture and hay (cropland for pasture, fallow and idle cropland, pastureland, and other hay), alfalfa, sorghum, non-wheat grains, tobacco, peanuts, sugarcane, sugar beets, and other miscellaneous crops [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"GssJpkAa","properties":{"formattedCitation":"(Baker and Stone 2015; Stone 2013; Thelin and Stone 2013)","plainCitation":"(Baker and Stone 2015; Stone 2013; Thelin and Stone 2013)","noteIndex":0},"citationItems":[{"id":2414,"uris":["http://zotero.org/users/local/opHgB1Dy/items/UTJNBZWL"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/UTJNBZWL"],"itemData":{"id":2414,"type":"report","title":"Estimation of Annual Agricultural Pesticide Use for Counties of the Conterminous United States, 1992–2009","URL":"https://pubs.usgs.gov/sir/2013/5009/","author":[{"family":"Thelin","given":"Gail P"},{"family":"Stone","given":"Wesley W"}],"accessed":{"date-parts":[["2019",7,20]]},"issued":{"date-parts":[["2013"]]}}},{"id":2416,"uris":["http://zotero.org/users/local/opHgB1Dy/items/CKJ6QWHE"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/CKJ6QWHE"],"itemData":{"id":2416,"type":"report","title":"Estimated Annual Agricultural Pesticide Use for Counties of the Conterminous United States, 1992–2009","URL":"https://pubs.usgs.gov/ds/752/","author":[{"family":"Stone","given":"Wesley W"}],"accessed":{"date-parts":[["2019",7,20]]},"issued":{"date-parts":[["2013"]]}}},{"id":2467,"uris":["http://zotero.org/users/local/opHgB1Dy/items/SFVBG7EQ"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/SFVBG7EQ"],"itemData":{"id":2467,"type":"report","number":"Data Series 907","title":"Estimated Annual Agricultural Pesticide Use for Counties of the Conterminous United States, 2008–12","URL":"https://pubs.usgs.gov/ds/0907/","author":[{"family":"Baker","given":"Nancy T"},{"family":"Stone","given":"Wesley W"}],"accessed":{"date-parts":[["2019",7,20]]},"issued":{"date-parts":[["2015"]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}].

We characterized estimated U.S. triazole fungicide usage stratified by year, specific compounds, crop type, and geographical location. To aid in interpretation, we used mean of the low and high annual agricultural pesticide estimates rather than presenting each separately. All analysis was completed in R (Version 1.0.143, RStudio) and maps were created in ArcGIS (ArcGIS Desktop 10.5.1, Esri Inc.).

Results

Estimated triazole fungicide use changed little between 1992 (428 metric tons) and 2006 (539 metric tons) (Figure 1). Since 2006, use increased substantially, reaching 2,880 metric tons in 2016, an increase of 434%. Triazole use by compound differed over time (Figure 2B, Supplemental Table 2). Use of propiconazole and tebuconazole, the most widely used fungicides in 2016, increased little from 1992 to 2006, whereas use increased by 366% for propiconazole and 229% for tebuconazole during 2006–2016. First use of three newer triazoles difenoconazole, metconazole, and prothiconazole was reported after 2006, and usage increased to a total of 732 metric tons in 2016. In contrast, estimated use of myclobutanil and triadimefon decreased during 1992–2016 (Figure 2B, Supplemental Table 2).

Triazole fungicide use by crop type also changed substantially over time (Figure 2A, Table 1). During 1992–2005, the primary use was on wheat, orchards and grapes, and other crops. Use on wheat began to increase markedly in 2007, with use increasing 683% during 2006–2016, resulting in the highest use amongst all crops in 2016 (1253 metric tons). Use on corn and soybeans also increased dramatically, with use on corn growing from 0 to 437 metric tons during 2006–2016, while use on soybeans increased from 61 to 361 metric tons. Use on other crops, rice, vegetables, and cotton increased steadily over time but at a slower rate. Use on orchards and grapes remained relatively constant (Figure 2A, Table 1).

The geographical distribution of triazole fungicide use shifted as use by crop type changed over time (Figure 3, Supplemental Table 3). The two states with the highest use during 2012–2016, North Dakota

(1,800 metric tons) and Georgia (1,008 metric tons), also had the largest increase during 1992–1996. This was primarily due to application on wheat in North Dakota and other crops, such as peanuts, in Georgia (Supplemental Figure 1). Although California had the third highest usage during 2012–2016 (711 metric tons), application increased <50% from 1992–1996; triazoles were used primarily on orchards and grapes. The geographic shift is apparent as triazole use increased in the Midwest with wheat, corn, and soybeans (Supplemental Figure 1).

Discussion

Overall U.S. triazole fungicide use in agriculture changed little during 1992–2005 increased >4-fold during 2006–2016 based on USGS estimates. Although estimated triazole usage increased in nearly every crop type and state over the period, the increase occurred primarily in wheat, corn, soybeans, and other crops in the Midwest and Southeast. These increases may have implications for triazole resistance in pathogenic fungi for humans, particularly in *A. fumigatus* based on evidence from Europe and elsewhere [ADDIN ZOTERO_ITEM CSL_CITATION
{"citationID":"W65En9PV","properties":{"formattedCitation":"(Bueid et al. 2010; Leli\\uc0\\u232{}vre et al. 2013; Resendiz-Sharpe et al. 2019)","plainCitation":"(Bueid et al. 2010; Lelièvre et al. 2013; Resendiz-Sharpe et al. 2019)","noteIndex":0},"citationItems":[{"id":3032,"uris":["http://zotero.org/users/local/opHgB1Dy/items/2M7K6H7Y"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/2M7K6H7Y"],"itemData":{"id":3032,"type":"article-journal","abstract":"AbstractBackground. Increasing resistance of Aspergillus fumigatus to triazoles in high-risk populations is a concern. Its impact on mortality is not well unde","container-title":"Journal of Antimicrobial Chemotherapy","DOI":"10.1093/jac/dkz258","ISSN":"0305-7453","issue":"9","journalAbbreviation":"J

Antimicrob Chemother","language":"en","page":"2759-2766","source":"academic.oup.com","title":"Prevalence of voriconazole-resistant invasive aspergillosis and its impact on mortality in haematology patients","volume":"74","author":[{"family":"Resendiz-Sharpe","given":"Agustin"},{"family":"Mercier","given":"Toine"},{"family":"Lestrade","given":"Pieter P. A."},{"family":"Beek","given":"Martha T.","non-dropping-particle":"van der"},{"family":"Borne","given":"Peter A.","non-dropping-particle":"von dem"},{"family":"Cornelissen","given":"Jan J."},{"family":"De Kort","given":"Elizabeth"},{"family":"Rijnders","given":"Bart J. A."},{"family":"Schauwvlieghe","given":"Alexander F. A. D."},{"family":"Verweij","given":"Paul E."},{"family":"Maertens","given":"Johan"},{"family":"Lagrou","given":"Katrien"}],"issued":{"date-parts":[["2019",9,1]]}},{"id":3037,"uris":["http://zotero.org/users/local/opHgB1Dy/items/7GAM2CCR"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/7GAM2CCR"],"itemData":{"id":3037,"type":"article-journal","abstract":"Azole resistance has appeared recently in Aspergillus fumigatus and increased dangerously in the last decade. The main resistance mechanism is a point mutation of CYP51A, the gene encoding 14α-sterol demethylase, the target enzyme of azole antifungal drugs. This mutation can induce resistance to itraconazole alone or multi-azole resistance. CYP51A mutation can occur in two cases. The first usually concerns patients receiving long-term azole therapy, most of the time for chronic aspergillosis, and involves a wide range of mutations. The second is due to the use of azole fungicides in agriculture. The latter favors a single mutagenesis event: a substitution of leucine for histidine at codon 98 and the tandem repeat of a 34-base pair tandem sequence in the CYP51A gene promoter region. This confers cross-resistance to all azole antifungal drugs. This emerging and environmentally linked issue is of growing concern for the management of antifungal therapy. This mechanism of resistance was first described in the Netherlands and is now reported worldwide. It may have become the leading mechanism of azole resistance in A. fumigatus. Azoles are major agents for the treatment of aspergillosis, and the only oral antifungals. Infection with antifungal-resistant strains is correlated with treatment failure. This emerging phenomenon stresses the urgent need for new preventive strategies (controlled use of antifungals and azole prophylaxis), new diagnostic strategies (early detection of resistance), and new therapeutic strategies in the management of A. fumigatus infections.\nRésumé\nLa résistance d'Aspergillus fumigatus aux azolés est apparue récemment et est en dangereuse augmentation depuis dix ans. Son principal mécanisme est lié à la mutation du gène CYP51A codant pour l'enzyme 14-α-déméthylase, cible des azolés. Celle-ci peut conduire à une résistance touchant l'itraconazole seul ou l'ensemble des molécules triazolées. Deux situations peuvent engendrer des mutations du gène CYP51A. La première apparaît classiquement chez le patient traité par azolés au long cours – le plus souvent pour une aspergillose chronique – et fait intervenir une grande variété de mutations. La seconde, émergente, est due à l'utilisation de fongicides azolés en agriculture et confère une résistance croisée à tous les azolés. Cette dernière repose sur la substitution d'une leucine par l'histidine au codon 98 du gène CYP51A, associée à la répétition en tandem d'une séquence de 34 paires de bases dans la région promotrice du gène (TR/L98H). Initialement décrit aux Pays-Bas, ce type de résistance diffuse maintenant à travers le monde et pourrait même être devenu, la principale source de résistance d'A. fumigatus aux azolés. Les azolés tiennent une place majeure dans le traitement des infections aspergillaires et représentent la seule classe utilisable par voie orale. Une infection à isolat résistant étant directement associée à l'échec thérapeutique, l'émergence de ce phénomène implique la mise en place de nouvelles stratégies à la fois préventives (maîtrise de l'utilisation des fongicides et des prophylaxies par azolés), diagnostiques (détection précoce de la résistance), et thérapeutiques dans la gestion des infections à A. fumigatus.","container-title":"Médecine et Maladies Infectieuses","DOI":"10.1016/j.medmal.2013.02.010","ISSN":"0399-077X","issue":"4","journalAbbreviation":"Médecine et Maladies Infectieuses","language":"en","page":"139-145","source":"ScienceDirect","title":"Azole resistant

Aspergillus fumigatus: An emerging problem","title-short":"Azole resistant Aspergillus fumigatus","volume":"43","author":[{"family":"Lelièvre","given":"L."},{"family":"Groh","given":"M."},{"family":"Angebault","given":"C."},{"family":"Maherault","given":"A. -C."},{"family":"Didier","given":"E."},{"family":"Bougnoux","given":"M. -E."}],"issued":{"date-parts":[["2013",4,1]]}},{"id":3040,"uris":["http://zotero.org/users/local/opHgB1Dy/items/YC9H5WZR"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/YC9H5WZR"],"itemData":{"id":3040,"type":"article-journal","abstract":"AbstractObjectives. Resistance to azole antifungal drugs in Aspergillus fumigatus is now a major clinical problem in some locations. Here we update our previou","container-title":"Journal of Antimicrobial Chemotherapy","DOI":"10.1093/jac/dkq279","ISSN":"0305-7453","issue":"10","journalAbbreviation":"J Antimicrob Chemother","language":"en","page":"2116-2118","source":"academic.oup.com","title":"Azole antifungal resistance in Aspergillus fumigatus: 2008 and 2009","title-short":"Azole antifungal resistance in Aspergillus fumigatus","volume":"65","author":[{"family":"Bueid","given":"Ahmed"},{"family":"Howard","given":"Susan J."},{"family":"Moore","given":"Caroline B."},{"family":"Richardson","given":"Malcolm D."},{"family":"Harrison","given":"Elizabeth"},{"family":"Bowyer","given":"Paul"},{"family":"Denning","given":"David W."}],"issued":{"date-parts":[["2010",10,1]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}]. Given that resistance mutations previously associated with environmental triazole use have recently been detected in U.S. patient and environmental *A. fumigatus* isolates, additional study of the role of agricultural fungicides is warranted.

Several factors may explain the dramatic increase in U.S. triazole fungicide use after 2006, including increased corn production in response to higher prices, increased plant diseases in certain regions, ability to use new fungicides on field crops, and marketing of fungicides for use on field crops [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"6PrtC6MT","properties":{"formattedCitation":"(Mueller et al.; Wise and Mueller 2011)","plainCitation":"(Mueller et al.; Wise and Mueller 2011)","noteIndex":0},"citationItems":[{"id":3241,"uris":["http://zotero.org/users/local/opHgB1Dy/items/Z8YX3R5D"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/Z8YX3R5D"],"itemData":{"id":3241,"type":"book","ISBN":"978-0-89054-506-5","publisher":"APS Publications","title":"Fungicides for Field Crops | Mycology","URL":"https://apsjournals.apsnet.org/doi/book/10.1094/9780890545065","author":[{"family":"Mueller","given":"Daren S."},{"family":"Wise","given":"Kiersten A."},{"family":"Dufault","given":"Nicholas S."},{"family":"Bradley","given":"Carl A."},{"family":"Chilvers","given":"Martin I."}],"accessed":{"date-parts":[["2019",12,13]]}}},{"id":2841,"uris":["http://zotero.org/users/local/opHgB1Dy/items/MB62XFGP"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/MB62XFGP"],"itemData":{"id":2841,"type":"article-journal","container-title":"APSnet Feature Articles","DOI":"10.1094/APSnetFeature-2011-0531","ISSN":"2153-0297","journalAbbreviation":"APSnet Features","language":"en","source":"DOI.org (Crossref)","title":"Are Fungicides No Longer Just For Fungi? An Analysis of Foliar Fungicide Use in Corn","title-short":"Are Fungicides No Longer Just For Fungi?","URL":"http://www.apsnet.org/publications/apsnetfeatures/Pages/fungicide.aspx","author":[{"family":"Wise","given":"Kiersten"},{"family":"Mueller","given":"Daren"}],"accessed":{"date-parts":[["2019",9,1]]},"issued":{"date-parts":[["2011"]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}]. For example, when soybean rust caused by the fungus *Phakopsora pachyrhizi* was first identified in the United States in 2004, several fungicides were registered or granted emergency exemptions for treatment of soybeans, including myclobutanil, propiconazole, tebuconazole, and tetraconazole [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"AmaC69IK","properties":{"formattedCitation":"(Battaglin et al. 2011; Sconyers et al.; Wise and Mueller 2011)","plainCitation":"(Battaglin et al. 2011; Sconyers et al.; Wise and Mueller

2011)","noteIndex":0},"citationItems":[{"id":2642,"uris":["http://zotero.org/users/local/opHgB1Dy/item
s/2DEC3MMT"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/2DEC3MMT"],"itemData":{"id":2
642,"type":"article-journal","container-title":"Water, Air, & Soil Pollution","DOI":"10.1007/s11270-010-
0643-2","ISSN":"0049-6979, 1573-2932","issue":"1-4","journalAbbreviation":"Water Air Soil
Pollut","language":"en","page":"307-322","source":"DOI.org (Crossref)","title":"Occurrence of
Azoxystrobin, Propiconazole, and Selected Other Fungicides in US Streams, 2005–
2006","volume":"218","author":[{"family":"Battaglin","given":"William
A."},{"family":"Sandstrom","given":"Mark W."},{"family":"Kuivila","given":"Kathryn
M."},{"family":"Kolpin","given":"Dana W."},{"family":"Meyer","given":"Michael T."}],"issued":{"date-
parts":[["2011",6]]}}},{"id":3208,"uris":["http://zotero.org/users/local/opHgB1Dy/items/C6GQVARB"],"u
ri":["http://zotero.org/users/local/opHgB1Dy/items/C6GQVARB"],"itemData":{"id":3208,"type":"report"
,"abstract":"APSnet Features. January, 2006...Dr. Layla E. Sconyers(Corresponding
author: lsconyrs@uga.edu)Department of Plant PathologyUniversity of GeorgiaCoastal Plain Experiment
StationTifton, GA 31793 Dr. Robert C. KemeraitDepartment of Plant PathologyUniversity of GeorgiaRural
Development CenterTifton, GA 31793 Mr. Jason...","language":"en-US","title":"Asian Soybean Rust
Development in 2005: A Perspective from the Southeastern United States","title-short":"Asian Soybean
Rust Development in
2005","URL":"https://www.apsnet.org/edcenter/apsnetfeatures/Pages/SoybeanRustDev.aspx","author"
:[{"family":"Sconyers","given":"Layla E."},{"family":"Kemerait","given":"Robert
C."},{"family":"Brock","given":"Jason"},{"family":"Phillips","given":"Daniel
V."},{"family":"Jost","given":"Philip H."},{"family":"Sikora","given":"Edward J."},{"family":"Gutierrez-
Estrada","given":"Arcenio"},{"family":"Mueller","given":"John D."},{"family":"Marois","given":"James
J."},{"family":"Wright","given":"David L."},{"family":"Harmon","given":"Carrie L."}],"accessed":{"date-
parts":[["2019",12,7]]}}},{"id":2841,"uris":["http://zotero.org/users/local/opHgB1Dy/items/MB62XFGP"]

,"uri":["http://zotero.org/users/local/opHgB1Dy/items/MB62XFGP"],"itemData":{"id":2841,"type":"article-journal","container-title":"APSnet Feature Articles","DOI":"10.1094/APSnetFeature-2011-0531","ISSN":"2153-0297","journalAbbreviation":"APSnet Features","language":"en","source":"DOI.org (Crossref)","title":"Are Fungicides No Longer Just For Fungi? An Analysis of Foliar Fungicide Use in Corn","title-short":"Are Fungicides No Longer Just For Fungi?","URL":"http://www.apsnet.org/publications/apsnetfeatures/Pages/fungicide.aspx","author":[{"family":"Wise","given":"Kiersten"},{"family":"Mueller","given":"Daren"}],"accessed":{"date-parts":[["2019",9,1]]},"issued":{"date-parts":[["2011"]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}]. Another class of fungicides called strobilurins have been marketed to increase soybean and corn yield, frequently in combination with triazoles [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"BVQvKnUU","properties":{"formattedCitation":"(Swoboda and Pedersen 2009; Wise and Mueller 2011)","plainCitation":"(Swoboda and Pedersen 2009; Wise and Mueller 2011)","noteIndex":0},"citationItems":[{"id":2841,"uris":["http://zotero.org/users/local/opHgB1Dy/items/MB62XFGP"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/MB62XFGP"],"itemData":{"id":2841,"type":"article-journal","container-title":"APSnet Feature Articles","DOI":"10.1094/APSnetFeature-2011-0531","ISSN":"2153-0297","journalAbbreviation":"APSnet Features","language":"en","source":"DOI.org (Crossref)","title":"Are Fungicides No Longer Just For Fungi? An Analysis of Foliar Fungicide Use in Corn","title-short":"Are Fungicides No Longer Just For Fungi?","URL":"http://www.apsnet.org/publications/apsnetfeatures/Pages/fungicide.aspx","author":[{"family":"Wise","given":"Kiersten"},{"family":"Mueller","given":"Daren"}],"accessed":{"date-parts":[["2019",9,1]]},"issued":{"date-parts":[["2011"]]}}},{"id":3067,"uris":["http://zotero.org/users/local/opHgB1Dy/items/22IK8Y9R"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/22IK8Y9R"],"itemData":{"id":3067,"type":"article-

journal","container-title":"Agronomy Journal;
Madison","ISSN":"00021962","issue":"2","language":"English","page":"352-356","source":"ProQuest","title":"Effect of Fungicide on Soybean Growth and Yield","volume":"101","author":[{"family":"Swoboda","given":"Catherine"},{"family":"Pedersen","given":"Palle"}],"issued":{"date-parts":[["2009",4]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}]. Fungicides are also used preemptively in what are informally called “insurance applications,” when they are added to spray tanks being used to apply other pesticides like herbicides or insecticides [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"IY3ihf66","properties":{"formattedCitation":"(DiFonzo 2012)","plainCitation":"(DiFonzo 2012)","noteIndex":0},"citationItems":[{"id":3073,"uris":["http://zotero.org/users/local/opHgB1Dy/items/XLUQ4RF8"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/XLUQ4RF8"],"itemData":{"id":3073,"type":"webpage","abstract":"Some growers may be considering preventative applications of fungicides and insecticides to field crops. But just what are the impacts of preventative or insurance pesticide applications on insect or mite control?","container-title":"MSU Extension","language":"en","title":"The hidden costs of insurance pesticide applications to field crops","URL":"https://www.canr.msu.edu/news/the_hidden_costs_of_insurance_pesticide_applications_to_field_crops","author":[{"family":"DiFonzo","given":"Christina"}],"accessed":{"date-parts":[["2019",10,23]]},"issued":{"date-parts":[["2012",6,21]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}]. More research may be helpful to understand the reasons behind the large increases in triazole fungicides.

Because both triazoles and *A. fumigatus* can move long distances in the environment, potential effects on resistant infections should be considered beyond the sites of application. Triazoles can persist and

travel in the environment and have been detected outside the site of application in surface waters across the country [ADDIN ZOTERO_ITEM CSL_CITATION
{"citationID":"YyE6C9I9","properties":{"formattedCitation":"(Battaglin et al. 2011; Nowell et al. 2018; Sanders et al. 2018; Smalling and Orlando 2011)","plainCitation":"(Battaglin et al. 2011; Nowell et al. 2018; Sanders et al. 2018; Smalling and Orlando 2011)","noteIndex":0},"citationItems":[{"id":2642,"uris":["http://zotero.org/users/local/opHgB1Dy/items/2DEC3MMT"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/2DEC3MMT"],"itemData":{"id":2642,"type":"article-journal","container-title":"Water, Air, & Soil Pollution","DOI":"10.1007/s11270-010-0643-2","ISSN":"0049-6979, 1573-2932","issue":"1-4","journalAbbreviation":"Water Air Soil Pollut","language":"en","page":"307-322","source":"DOI.org (Crossref)","title":"Occurrence of Azoxystrobin, Propiconazole, and Selected Other Fungicides in US Streams, 2005–2006","volume":"218","author":[{"family":"Battaglin","given":"William A."},{"family":"Sandstrom","given":"Mark W."},{"family":"Kuivila","given":"Kathryn M."},{"family":"Kolpin","given":"Dana W."},{"family":"Meyer","given":"Michael T."}],"issued":{"date-parts":[["2011",6]]}}},{"id":3268,"uris":["http://zotero.org/users/local/opHgB1Dy/items/XE94GIRR"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/XE94GIRR"],"itemData":{"id":3268,"type":"article-journal","container-title":"Science of The Total Environment","DOI":"10.1016/j.scitotenv.2017.06.156","ISSN":"00489697","journalAbbreviation":"Science of The Total Environment","language":"en","page":"1469-1488","source":"DOI.org (Crossref)","title":"Complex mixtures of dissolved pesticides show potential aquatic toxicity in a synoptic study of Midwestern U.S. streams","volume":"613-614","author":[{"family":"Nowell","given":"Lisa H."},{"family":"Moran","given":"Patrick W."},{"family":"Schmidt","given":"Travis S."},{"family":"Norman","given":"Julia E."},{"family":"Nakagaki","given":"Naomi"},{"family":"Shoda","given":"Megan

E."},{"family":"Mahler","given":"Barbara J."},{"family":"Van Metre","given":"Peter C."},{"family":"Stone","given":"Wesley W."},{"family":"Sandstrom","given":"Mark W."},{"family":"Hladik","given":"Michelle L."}],"issued":{"date-parts":[["2018",2]]}}},{"id":3271,"uris":["http://zotero.org/users/local/opHgB1Dy/items/ARSYCVBU"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/ARSYCVBU"],"itemData":{"id":3271,"type":"report","genre":"Data Series","language":"en","number":"Data Series 1088","source":"DOI.org (Crossref)","title":"Detections of Current-Use Pesticides at 12 Surface Water Sites in California During a 2-Year Period Beginning in 2015","author":[{"family":"Sanders","given":"Corey J."},{"family":"Orlando","given":"James L."},{"family":"Hladik","given":"Michelle L."}],"issued":{"date-parts":[["2018"]]}}},{"id":3263,"uris":["http://zotero.org/users/local/opHgB1Dy/items/IDZ3FINJ"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/IDZ3FINJ"],"itemData":{"id":3263,"type":"report","genre":"Data Series","language":"en","number":"Data Series 600","source":"DOI.org (Crossref)","title":"Occurrence of Pesticides in Surface Water and Sediments from Three Central California Coastal Watersheds, 2008–09","author":[{"family":"Smalling","given":"Kelly L."},{"family":"Orlando","given":"James L."}],"issued":{"date-parts":[["2011"]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}]. Further, triazoles can be transported long distances in the atmosphere [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"DA2qEtB8","properties":{"formattedCitation":"(D\\uc0\\u233{}sert et al. 2018; Schummer et al. 2010)","plainCitation":"(Désert et al. 2018; Schummer et al. 2010)","noteIndex":0},"citationItems":[{"id":3269,"uris":["http://zotero.org/users/local/opHgB1Dy/items/T72RQNVT"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/T72RQNVT"],"itemData":{"id":3269,"type":"article-journal","abstract":"A total of 59 current-use pesticides were monitored in ambient air samples collected from February 2012 to December 2017, at two rural and six urban sites in Provence-

Alpes-Côte-d’Azur Region and Corsica, France. 45 of searched active substances were detected at least in one sample, at frequencies ranging from 0.1 to 98.6%. Among the most frequently detected pesticides, we found the herbicide Pendimethalin (64.6%), the fungicide Tebuconazole (65.9%), and the insecticides Chlorpyrifos (71.5%) and Lindane (98.6%). A wide range of atmospheric concentrations was measured from few pg m−3 to several hundreds of ng m−3, with a maximum concentration of 407.79 ng m−3 for Chlorpyrifos (Cavaillon, May 2012). 17 active substances exceeded an atmospheric concentration of 1 ng m−3 for at least one sample, including Folpet (147 times/162 detections), Chlorpyrifos (56/520), and Pendimethalin (29/464). The spatial distribution shows that pesticides were detected both in the eight rural and urban sampling sites, suggesting an atmospheric transport from agricultural areas to cities. Classifying the 8 sampling sites according to samples composition, two types of site were observed, those (Aléria, Arles, Avignon, Port-de-Bouc, and Toulon) where a majority of fungicides are found and those (Cannes, Cavaillon, and Nice) where insecticides are dominant. Long-term (6 years) monitoring shows a seasonally trend for each pesticide, depending on pest pressure. Inter-annual variation suggests a downward trend which is consistent with the regional sales data.","container-title":"Atmospheric Environment","DOI":"10.1016/j.atmosenv.2018.08.054","ISSN":"13522310","journalAbbreviation":"Atmospheric Environment","language":"en","page":"241-256","source":"DOI.org (Crossref)","title":"Spatial and temporal distribution of current-use pesticides in ambient air of Provence-Alpes-Côte-d’Azur Region and Corsica, France","volume":"192","author":[{"family":"Désert","given":"Marine"},{"family":"Ravier","given":"Sylvain"},{"family":"Gille","given":"Grégory"},{"family":"Quinapallo","given":"Angélina"},{"family":"Armengaud","given":"Alexandre"},{"family":"Pochet","given":"Gabrielle"},{"family":"Savelli","given":"Jean-Luc"},{"family":"Wortham","given":"Henri"},{"family":"Quivet","given":"Etienne"}],"issued":{"date-parts":[["2018",11]]}}},{"id":3265,"uris":["http://zotero.org/users/local/opHgB1Dy/items/5DHTT64E"],"u ri":["http://zotero.org/users/local/opHgB1Dy/items/5DHTT64E"],"itemData":{"id":3265,"type":"article-journal","abstract":"Atmospheric samples have been collected in Strasbourg between April 18 and May 29, 2007 and were analyzed for 71 current-use pesticides, of which 38 were detected. Average concentrations ranged from 0.09 ng mÀ3 for Fenarimol to 110.42 ng mÀ3 for Dimethachlor, which was slightly higher than the concentrations reported from other, comparable agricultural regions.","container-title":"Environmental Pollution","DOI":"10.1016/j.envpol.2009.08.019","ISSN":"02697491","issue":"2","journalAbbreviation":"Environmental Pollution","language":"en","page":"576-584","source":"DOI.org (Crossref)","title":"Temporal variations of concentrations of currently used pesticides in the atmosphere of Strasbourg, France","volume":"158","author":[{"family":"Schummer","given":"Claude"},{"family":"Mothiron","given":"Elodie"},{"family":"Appenzeller","given":"Brice M.R."},{"family":"Rizet","given":"Anne-Laure"},{"family":"Wennig","given":"Robert"},{"family":"Millet","given":"Maurice"}],"issued":{"date-parts":[["2010",2]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}] and residues have been detected in amphibians living in remote locations in the Sierra Nevada, dozens of miles downwind from where they were applied [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"xPH4y2kf","properties":{"formattedCitation":"(Smalling et al. 2013)","plainCitation":"(Smalling et al. 2013)","noteIndex":0},"citationItems":[{"id":2749,"uris":["http://zotero.org/users/local/opHgB1Dy/items/9H9N6RBL"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/9H9N6RBL"],"itemData":{"id":2749,"type":"article-journal","container-title":"Environmental Toxicology and Chemistry","DOI":"10.1002/etc.2308","ISSN":"07307268","issue":"9","journalAbbreviation":"Environ Toxicol Chem","language":"en","page":"2026-2034","source":"DOI.org (Crossref)","title":"Accumulation of pesticides in pacific chorus frogs (<i>Pseudacris regilla</i>) from California's Sierra Nevada

Mountains, USA: Pesticides residues in amphibians","title-short":"Accumulation of pesticides in pacific chorus frogs (<i>Pseudacris regilla</i>) from California's Sierra Nevada Mountains, USA","volume":"32","author":[{"family":"Smalling","given":"Kelly L."},{"family":"Fellers","given":"Gary M."},{"family":"Kleeman","given":"Patrick M."},{"family":"Kuivila","given":"Kathryn M."}],"issued":{"date-parts":[["2013",9]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}]. This mobility means that *A. fumigatus* growing in areas far from agricultural land may be exposed to triazoles, providing opportunity for resistance to develop. *A. fumigatus* spores, like spores of fungal plant pathogens, can travel long distances in the air [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"uXnUIZdh","properties":{"formattedCitation":"(Brown and Hovm\\uc0\\u248{}ller 2002)","plainCitation":"(Brown and Hovmøller 2002)","noteIndex":0},"citationItems":[{"id":3211,"uris":["http://zotero.org/users/local/opHgB1Dy/items/S22X9N6U"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/S22X9N6U"],"itemData":{"id":3211,"type":"article-journal","abstract":"Some of the most striking and extreme consequences of rapid, long-distance aerial dispersal involve pathogens of crop plants. Long-distance dispersal of fungal spores by the wind can spread plant diseases across and even between continents and reestablish diseases in areas where host plants are seasonally absent. For such epidemics to occur, hosts that are susceptible to the same pathogen genotypes must be grown over wide areas, as is the case with many modern crops. The strongly stochastic nature of long-distance dispersal causes founder effects in pathogen populations, such that the genotypes that cause epidemics in new territories or on cultivars with previously effective resistance genes may be atypical. Similar but less extreme population dynamics may arise from long-distance aerial dispersal of other organisms, including plants, viruses, and fungal pathogens of humans.","container-title":"Science","DOI":"10.1126/science.1072678","ISSN":"0036-8075, 1095-9203","issue":"5581","language":"en","note":"PMID: 12142520","page":"537-

541","source":"science.sciencemag.org","title":"Aerial Dispersal of Pathogens on the Global and Continental Scales and Its Impact on Plant Disease","volume":"297","author":[{"family":"Brown","given":"James K. M."},{"family":"Hovmøller","given":"Mogens S."}],"issued":{"date-parts":[["2002",7,26]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}]. Triazole-resistant *A. fumigatus* isolates with fungicide-associated TR mutations have been found inside the homes and in the yards of aspergillosis patients, in hospital gardens, and even in air samples taken from inside hospitals [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"a6Cna5yx","properties":{"formattedCitation":"(Chowdhary et al. 2014b; Lavergne et al. 2017; van der Linden et al. 2013)","plainCitation":"(Chowdhary et al. 2014b; Lavergne et al. 2017; van der Linden et al. 2013)","noteIndex":0},"citationItems":[{"id":2251,"uris":["http://zotero.org/users/local/opHgB1Dy/items/AXJVNPXJ"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/AXJVNPXJ"],"itemData":{"id":2251,"type":"article-journal","abstract":"Background. Azole resistance is an emerging problem in Aspergillus fumigatus and complicates the management of patients with Aspergillus-related diseases. Selection of azole resistance may occur through exposure to azole fungicides in the environment. In the Netherlands a surveillance network was used to investigate the epidemiology of resistance selection in A. fumigatus.\nMethods. Clinical A. fumigatus isolates were screened for azole resistance in 8 university hospitals using azole agar dilution plates. Patient information was collected using an online questionnaire and azole-resistant A. fumigatus isolates were analyzed using gene sequencing, susceptibility testing, and genotyping. Air sampling was performed to investigate the presence of resistant isolates in hospitals and domiciles.\nResults. Between December 2009 and January 2011, 1315 A. fumigatus isolates from 921 patients were screened. A new cyp51A-mediated resistance mechanism (TR46/Y121F/T289A) was observed in 21 azole-resistant isolates from 15 patients in 6 hospitals.

TR46/Y121F/T289A isolates were highly resistant to voriconazole (minimum inhibitory concentration ≥16 mg/L). Eight patients presented with invasive aspergillosis due to TR46/Y121F/T289A, and treatment failed in all 5 patients receiving primary therapy with voriconazole. TR46/Y121F/T289A Aspergillus fumigatus was recovered from 6 of 10 sampled environmental sites.\nConclusions. We describe the emergence and geographical migration of a voriconazole highly resistant A. fumigatus that was associated with voriconazole treatment failure in patients with invasive aspergillosis. Recovery of TR46/Y121F/T289A from the environment suggests an environmental route of resistance selection. Exposure of A. fumigatus to azole fungicides may facilitate the emergence of new resistance mechanisms over time, thereby compromising the use of azoles in the management of Aspergillus-related diseases.","container-title":"Clinical Infectious Diseases","DOI":"10.1093/cid/cit320","ISSN":"1537-6591, 1058-4838","issue":"4","language":"en","page":"513-520","source":"DOI.org (Crossref)","title":"Aspergillosis due to Voriconazole Highly Resistant Aspergillus fumigatus and Recovery of Genetically Related Resistant Isolates From Domiciles","volume":"57","author":[{"family":"Linden","given":"Jan W. M.","non-dropping-particle":"van der"},{"family":"Camps","given":"Simone M. T."},{"family":"Kampinga","given":"Greetje A."},{"family":"Arends","given":"Jan P. A."},{"family":"Debets-Ossenkopp","given":"Yvette J."},{"family":"Haas","given":"Pieter J. A."},{"family":"Rijnders","given":"Bart J. A."},{"family":"Kuijper","given":"Ed J."},{"family":"Tiel","given":"Frank H.","non-dropping-particle":"van"},{"family":"Varga","given":"János"},{"family":"Karawajczyk","given":"Anna"},{"family":"Zoll","given":"J."},{"family":"Melchers","given":"Willem J. G."},{"family":"Verweij","given":"Paul E."}],"issued":{"date-parts":[["2013",8,15]]}}},{"id":2806,"uris":["http://zotero.org/users/local/opHgB1Dy/items/ATYYTHBB"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/ATYYTHBB"],"itemData":{"id":2806,"type":"articl e-journal","abstract":"AbstractObjectives. Azole resistance in Aspergillus fumigatus isolates has been increasingly reported with variable prevalence worldwide and is challenging the","container-title":"Journal of Antimicrobial Chemotherapy","DOI":"10.1093/jac/dku259","ISSN":"0305-7453","issue":"11","journalAbbreviation":"J Antimicrob Chemother","language":"en","page":"2979-2983","source":"academic.oup.com","title":"Multi-azole-resistant Aspergillus fumigatus in the environment in Tanzania","volume":"69","author":[{"family":"Chowdhary","given":"Anuradha"},{"family":"Sharma","given":"Cheshta"},{"family":"Boom","given":"Mara","non-dropping-particle":"van den"},{"family":"Yntema","given":"Jan Bart"},{"family":"Hagen","given":"Ferry"},{"family":"Verweij","given":"Paul E."},{"family":"Meis","given":"Jacques F."}],"issued":{"date-parts":[["2014",11,1]]}}},{"id":2502,"uris":["http://zotero.org/users/local/opHgB1Dy/items/XTF4TARJ"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/XTF4TARJ"],"itemData":{"id":2502,"type":"article-journal","container-title":"Clinical Infectious Diseases","DOI":"10.1093/cid/ciw664","ISSN":"1058-4838, 1537-6591","issue":"1","journalAbbreviation":"Clin Infect Dis.","language":"en","page":"76-78","source":"DOI.org (Crossref)","title":"Home Environment as a Source of Life-Threatening Azole-Resistant <i>Aspergillus fumigatus</i> in Immunocompromised Patients: Table 1.","title-short":"Home Environment as a Source of Life-Threatening Azole-Resistant <i>Aspergillus fumigatus</i> in Immunocompromised Patients","volume":"64","author":[{"family":"Lavergne","given":"Rose-Anne"},{"family":"Chouaki","given":"Taieb"},{"family":"Hagen","given":"Ferry"},{"family":"Toublanc","given":"Bénédicte"},{"family":"Dupont","given":"Hervé"},{"family":"Jounieaux","given":"Vincent"},{"family":"Meis","given":"Jacques F."},{"family":"Morio","given":"Florent"},{"family":"Le Pape","given":"Patrice"}],"issued":{"date-parts":[["2017",1,1]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-

citation.json"}]. While the location where resistance mutations arose in these isolates is not known, all were found in settings unlikely to be exposed to triazole fungicides. Taken together, these observations suggest that both triazole fungicides and spores of triazole-resistant *Aspergillus* can travel far from the site of fungicide application, putting immunocompromised patients at risk of resistant infections even in areas without known agricultural triazole fungicide use.

Important parallels can be drawn between challenges with agricultural use of medically important triazoles and agricultural use of medically important antibacterial drugs. In recent years, antibiotic use has been brought under veterinary oversight through the Veterinary Feed Directive [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"q4iv8EC4","properties":{"formattedCitation":"(Center for Veterinary Medicine 2019a, 2019b)","plainCitation":"(Center for Veterinary Medicine 2019a, 2019b)","noteIndex":0},"citationItems":[{"id":3193,"uris":["http://zotero.org/users/local/opHgB1Dy/items/QNH8AN93"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/QNH8AN93"],"itemData":{"id":3193,"type":"webpage","abstract":"Recommended approach for assessing the safety of antimicrobial new animal drugs regarding their microbiological effects on bacteria of human health concern.","container-title":"U.S. Food and Drug Administration","language":"en","title":"CVM GFI #152 Evaluating the Safety of Antimicrobial New Animal Drugs with Regard to Their Microbiological Effects on Bacteria of Human Health Concern","URL":"http://www.fda.gov/regulatory-information/search-fda-guidance-documents/cvm-gfi-152-evaluating-safety-antimicrobial-new-animal-drugs-regard-their-microbiological-effects","author":[{"family":"Center for Veterinary Medicine","given":""}],"accessed":{"date-parts":[["2019",12,5]]},"issued":{"date-parts":[["2019",4,16]]}}},{"id":3196,"uris":["http://zotero.org/users/local/opHgB1Dy/items/2H2TCLBC"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/2H2TCLBC"],"itemData":{"id":3196,"type":"webpage","abstract":"A framework for voluntary adoption of practices to ensure the appropriate or judicious use of medically important antimicrobial drugs in food-producing animals.","container-title":"U.S. Food and Drug Administration","language":"en","title":"CVM GFI #209 The Judicious Use of Medically Important Antimicrobial Drugs in Food-Producing Animals","URL":"http://www.fda.gov/regulatory-information/search-fda-guidance-documents/cvm-gfi-209-judicious-use-medically-important-antimicrobial-drugs-food-producing-animals","author":[{"literal":"Center for Veterinary Medicine"}],"accessed":{"date-parts":[["2019",12,5]]},"issued":{"date-parts":[["2019",4,16]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}]. Given that greater use of an antimicrobial tends to lead to greater levels of antimicrobial resistance, and that triazole-resistant infections are emerging in plants, greater triazole resistance in human pathogens may emerge as well [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"kkFRNWjd","properties":{"formattedCitation":"(Chowdhary et al. 2013)","plainCitation":"(Chowdhary et al. 2013)","noteIndex":0},"citationItems":[{"id":2344,"uris":["http://zotero.org/users/local/opHgB1Dy/items/XYR67Y8C"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/XYR67Y8C"],"itemData":{"id":2344,"type":"article-journal","container-title":"PLOS Pathogens","DOI":"10.1371/journal.ppat.1003633","ISSN":"1553-7374","issue":"10","journalAbbreviation":"PLOS Pathogens","language":"en","page":"e1003633","source":"PLoS Journals","title":"Emergence of Azole-Resistant Aspergillus fumigatus Strains due to Agricultural Azole Use Creates an Increasing Threat to Human Health","volume":"9","author":[{"family":"Chowdhary","given":"Anuradha"},{"family":"Kathuria","given":"Shallu"},{"family":"Xu","given":"Jianping"},{"family":"Meis","given":"Jacques F."}],"issued":{"date-parts":[["2013",10,24]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}].

Although detection of TR_{34} and TR_{46} has been limited in the United States [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"ClOMdMJM","properties":{"formattedCitation":"(Beer 2018)","plainCitation":"(Beer 2018)","noteIndex":0},"citationItems":[{"id":2169,"uris":["http://zotero.org/users/local/opHgB1Dy/items/J83LUV2C"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/J83LUV2C"],"itemData":{"id":2169,"type":"article-journal","abstract":"The environmental mold Aspergillus fumigatus is the primary cause of invasive aspergillosis. In patients with high-risk conditions, including stem cell and organ transplant recipients, mortality ...","container-title":"MMWR. Morbidity and Mortality Weekly Report","DOI":"10.15585/mmwr.mm6738a5","ISSN":"0149-21951545-861X","journalAbbreviation":"MMWR Morb Mortal Wkly Rep","language":"en-us","source":"www.cdc.gov","title":"Multidrug-Resistant Aspergillus fumigatus Carrying Mutations Linked to Environmental Fungicide Exposure — Three States, 2010–2017","URL":"https://www.cdc.gov/mmwr/volumes/67/wr/mm6738a5.htm","volume":"67","author":[{"family":"Beer","given":"Karlyn D."}],"accessed":{"date-parts":[["2019",7,5]]},"issued":{"date-parts":[["2018"]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}], surveillance, reporting, and susceptibility testing for *A. fumigatus* infections are not routinely conducted, suggesting that such infections are likely more widespread. For example, only 62% of the infectious disease doctors surveyed through the Emerging Infections Network in the United States reported having access to susceptibility testing for *A. fumigatus*, and such tests were not routinely ordered. Nevertheless, physicians reported seeing resistance in the United States, with 19% observing any triazole resistance and 7% pan-resistance. Only 14% were aware of a possible link to environmental fungicide use [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"FEIETJiP","properties":{"formattedCitation":"(Walker et al.

2018)","plainCitation":"(Walker et al.
2018)","noteIndex":0},"citationItems":[{"id":429,"uris":["http://zotero.org/users/local/opHgB1Dy/items
/DTZQE9QY"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/DTZQE9QY"],"itemData":{"id":429,
"type":"article-journal","abstract":"Of 709 physicians, 348 were familiar with azole-resistant
Aspergillus\nfumigatus; of those treating case-patients, 21% lacked access to susceptibility testing.,
Infections caused by pan–azole-resistant Aspergillus fumigatus strains have emerged in Europe and
recently in the United States. Physicians specializing in infectious diseases reported observing pan–
azole-resistant infections and low rates of susceptibility testing, suggesting the need for wider-scale
testing.","container-title":"Emerging Infectious
Diseases","DOI":"10.3201/eid2401.170971","ISSN":"1080-
6040","issue":"1","journalAbbreviation":"Emerg Infect Dis","note":"PMID: 29261092\nPMCID:
PMC5749442","page":"111-113","source":"PubMed Central","title":"Recognition of Azole-Resistant
Aspergillosis by Physicians Specializing in Infectious Diseases, United
States","volume":"24","author":[{"family":"Walker","given":"Tiffany
A."},{"family":"Lockhart","given":"Shawn R."},{"family":"Beekmann","given":"Susan
E."},{"family":"Polgreen","given":"Philip
M."},{"family":"Santibanez","given":"Scott"},{"family":"Mody","given":"Rajal
K."},{"family":"Beer","given":"Karlyn D."},{"family":"Chiller","given":"Tom
M."},{"family":"Jackson","given":"Brendan R."}],"issued":{"date-
parts":[["2018",1]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-
citation.json"}]. In contrast, testing for resistance in *A. fumigatus* in Europe is more widespread. The European Centre for Disease Prevention and Control recommends triazole antifungal susceptibility testing on all clinical *A. fumigatus* isolates when starting antifungal therapy [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"REDg49Bc","properties":{"formattedCitation":"(Kleinkauf and European

Centre for Disease Prevention and Control 2013)","plainCitation":"(Kleinkauf and European Centre for Disease Prevention and Control 2013)","noteIndex":0},"citationItems":[{"id":1086,"uris":["http://zotero.org/users/local/opHgB1Dy/items/3628VSAE"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/3628VSAE"],"itemData":{"id":1086,"type":"book","collection-title":"Technical report / European Centre for Disease Prevention and Control","event-place":"Stockholm","ISBN":"978-92-9193-444-7","language":"en","note":"OCLC: 855553214","number-of-pages":"17","publisher":"ECDC [u.a.] Europäisches Zentrum für die Prävention und die Kontrolle von Krankheiten","publisher-place":"Stockholm","source":"Gemeinsamer Bibliotheksverbund ISBN","title":"Risk assessment on the impact of environmental usage of triazoles on the development and spread of resistance to medical triazoles in Aspergillus species","editor":[{"family":"Kleinkauf","given":"Niels"},{"literal":"European Centre for Disease Prevention and Control"}],"issued":{"date-parts":[["2013"]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}], and this type of guideline had already been adopted in countries such as the Netherlands. In the United States, standards exist for antifungal susceptibility testing, but limited awareness points to a better need for increased vigilance for resistance among clinicians treating patients with invasive aspergillosis, even if they are triazole-naïve.

Several limitations are inherent in this analysis. First, the USGS data incorporated extrapolation methods to estimate pesticide use at the county level, and some degree of error is expected. However, we compared fungicide usage across states for multiple years and did not examine county-level data, which would tend to mitigate this problem. In this analysis, we took the mean of the USGS low and high triazole, which is a simplification based on complex models. However, overall trends were present in both the low and high estimates, suggesting this method is sufficient for this descriptive analysis. Second, we did not adjust triazole usage by units of arable land by state or crop, although these may be areas of further study. Finally, although available evidence points to environmental fungicide use as the main driver of TR-based triazole resistance in *A. fumigatus*, direct associations between quantity of agricultural fungicide use and resistant human infections in the United States have not been established.

In the United States, an opportunity may exist to intervene early before *A. fumigatus* resistance becomes a larger clinical problem. Research and partnerships are helpful in several areas. First, more robust laboratory-based surveillance for *A. fumigatus* infections [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"mR4dr11s","properties":{"formattedCitation":"(Verweij et al. 2016b)","plainCitation":"(Verweij et al. 2016b)","noteIndex":0},"citationItems":[{"id":2496,"uris":["http://zotero.org/users/local/opHgB1Dy/items/NF3L6M5Q"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/NF3L6M5Q"],"itemData":{"id":2496,"type":"article-journal","abstract":"Abstract. Azole resistance is a growing concern with Aspergillus fumigatus, and may cause increased mortality in patients with azole-resistant invasive aspergi","container-title":"Journal of Antimicrobial Chemotherapy","DOI":"10.1093/jac/dkw259","ISSN":"0305-7453","issue":"8","journalAbbreviation":"J Antimicrob Chemother","language":"en","page":"2079-2082","source":"academic.oup.com","title":"Azole resistance surveillance in Aspergillus fumigatus: beneficial or biased?","title-short":"Azole resistance surveillance in Aspergillus fumigatus","volume":"71","author":[{"family":"Verweij","given":"Paul E."},{"family":"Lestrade","given":"Pieter P. A."},{"family":"Melchers","given":"Willem J. G."},{"family":"Meis","given":"Jacques F."}],"issued":{"date-parts":[["2016",8,1]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}], including systematic antifungal susceptibility testing, could better determine the burden of resistant infections, as well as geographic and temporal trends. Second, wider-scale environmental testing could assess the distribution of resistance in the environment. Third, interdisciplinary One Health partnerships could identify ways to mitigate resistance, including exploring alternative fungicides and integrated pest management [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"sMqum0rK","properties":{"formattedCitation":"(Chowdhary et al. 2013; Fisher et al. 2018)","plainCitation":"(Chowdhary et al. 2013; Fisher et al. 2018)","noteIndex":0},"citationItems":[{"id":2344,"uris":["http://zotero.org/users/local/opHgB1Dy/items/XYR67Y8C"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/XYR67Y8C"],"itemData":{"id":2344,"type":"article-journal","container-title":"PLOS Pathogens","DOI":"10.1371/journal.ppat.1003633","ISSN":"1553-7374","issue":"10","journalAbbreviation":"PLOS Pathogens","language":"en","page":"e1003633","source":"PLoS Journals","title":"Emergence of Azole-Resistant Aspergillus fumigatus Strains due to Agricultural Azole Use Creates an Increasing Threat to Human Health","volume":"9","author":[{"family":"Chowdhary","given":"Anuradha"},{"family":"Kathuria","given":"Shallu"},{"family":"Xu","given":"Jianping"},{"family":"Meis","given":"Jacques F."}],"issued":{"date-parts":[["2013",10,24]]}}},{"id":2435,"uris":["http://zotero.org/users/local/opHgB1Dy/items/Q2N46CIN"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/Q2N46CIN"],"itemData":{"id":2435,"type":"article-journal","abstract":"<p>The recent rate of emergence of pathogenic fungi that are resistant to the limited number of commonly used antifungal agents is unprecedented. The azoles, for example, are used not only for human and animal health care and crop protection but also in antifouling coatings and timber preservation. The ubiquity and multiple uses of azoles have hastened the independent evolution of resistance in many environments. One consequence is an increasing risk in human health care from naturally occurring opportunistic fungal pathogens that have acquired resistance to this broad class of chemicals. To avoid a global collapse in our ability to control fungal infections and to avoid critical failures in medicine and food security, we must improve our stewardship of extant chemicals, promote new antifungal discovery, and leverage emerging technologies for alternative solutions.</p>","container-title":"Science","DOI":"10.1126/science.aap7999","ISSN":"0036-8075, 1095-9203","issue":"6390","language":"en","note":"PMID: 29773744","page":"739-742","source":"science.sciencemag.org","title":"Worldwide emergence of resistance to antifungal drugs challenges human health and food security","volume":"360","author":[{"family":"Fisher","given":"Matthew C."},{"family":"Hawkins","given":"Nichola J."},{"family":"Sanglard","given":"Dominique"},{"family":"Gurr","given":"Sarah J."}],"issued":{"date-parts":[["2018",5,18]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}]. Dozens of fungicide classes are available in the United States, whereas only three antifungal classes are available to treat severe human infections. Finally, antifungal stewardship in human medicine plays an important role in judicious use of these limited and important medications, and hospital stewardship programs have been shown to reduce the burden of antimicrobial-resistant human infections [ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"On9KF0Fv","properties":{"formattedCitation":"(Ananda-Rajah et al. 2012 p.; Baur et al. 2017)","plainCitation":"(Ananda-Rajah et al. 2012 p.; Baur et al. 2017)","noteIndex":0},"citationItems":[{"id":3186,"uris":["http://zotero.org/users/local/opHgB1Dy/items/ATXCLAJE"],"uri":["http://zotero.org/users/local/opHgB1Dy/items/ATXCLAJE"],"itemData":{"id":3186,"type":"article-journal","abstract":"Purpose of reviewAntimicrobial stewardship (AMS) has overwhelmingly focussed on antibiotics while antifungal agents have been largely neglected despite the few published audits of antifungal drug use demonstrating clear deficiencies in prescribing behaviour. In this review, we outline not only the e","container-title":"Current Opinion in Infectious Diseases","DOI":"10.1097/QCO.0b013e32834e0680","ISSN":"0951-

7375","issue":"1","language":"ENGLISH","note":"PMID: 22123667","page":"107-
115","source":"insights.ovid.com","title":"The case for antifungal
stewardship","volume":"25","author":[{"family":"Ananda-
Rajah","given":"Michelle"},{"family":"Slavin","given":"Monica"},{"family":"Thursky","given":"Karin"}],"iss
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g/users/local/opHgB1Dy/items/TY9E3CF2"],"itemData":{"id":3189,"type":"article-
journal","abstract":"Background\nAntibiotic stewardship programmes have been shown to reduce antibiotic use and hospital costs. We aimed to evaluate evidence of the effect of antibiotic stewardship on the incidence of infections and colonisation with antibiotic-resistant bacteria.\nMethods\nFor this systematic review and meta-analysis, we searched PubMed, the Cochrane Database of Systematic Reviews, the Cochrane Central Register of Controlled Trials, and Web of Science for studies published from Jan 1, 1960, to May 31, 2016, that analysed the effect of antibiotic stewardship programmes on the incidence of infection and colonisation with antibiotic-resistant bacteria and Clostridium difficile infections in hospital inpatients. Two authors independently assessed the eligibility of trials and extracted data. Studies involving long-term care facilities were excluded. The main outcomes were incidence ratios (IRs) of target infections and colonisation per 1000 patient-days before and after implementation of antibiotic stewardship. Meta-analyses were done with random-effect models and heterogeneity was calculated with the I2 method.\nFindings\nWe included 32 studies in the meta-analysis, comprising 9 056 241 patient-days and 159 estimates of IRs. Antibiotic stewardship programmes reduced the incidence of infections and colonisation with multidrug-resistant Gram-negative bacteria (51% reduction; IR 0·49, 95% CI 0·35–0·68; p<0·0001), extended-spectrum β-lactamase-producing Gram-negative bacteria (48%; 0·52, 0·27–0·98; p=0·0428), and meticillin-resistant Staphylococcus aureus (37%; 0·63, 0·45–0·88; p=0·0065), as well as the incidence of C difficile infections

(32%; 0·68, 0·53–0·88; p=0·0029). Antibiotic stewardship programmes were more effective when implemented with infection control measures (IR 0·69, 0·54–0·88; p=0·0030), especially hand-hygiene interventions (0·34, 0·21–0·54; p<0·0001), than when implemented alone. Antibiotic stewardship did not affect the IRs of vancomycin-resistant enterococci and quinolone-resistant and aminoglycoside-resistant Gram-negative bacteria. Significant heterogeneity between studies was detected, which was partly explained by the type of interventions and co-resistance patterns of the target bacteria.\nInterpretation\nAntibiotic stewardship programmes significantly reduce the incidence of infections and colonisation with antibiotic-resistant bacteria and C difficile infections in hospital inpatients. These results provide stakeholders and policy makers with evidence for implementation of antibiotic stewardship interventions to reduce the burden of infections from antibiotic-resistant bacteria.\nFunding\nGerman Center for Infection Research.","container-title":"The Lancet Infectious Diseases","DOI":"10.1016/S1473-3099(17)30325-0","ISSN":"1473-3099","issue":"9","journalAbbreviation":"The Lancet Infectious Diseases","language":"en","page":"990-1001","source":"ScienceDirect","title":"Effect of antibiotic stewardship on the incidence of infection and colonisation with antibiotic-resistant bacteria and Clostridium difficile infection: a systematic review and meta-analysis","title-short":"Effect of antibiotic stewardship on the incidence of infection and colonisation with antibiotic-resistant bacteria and Clostridium difficile infection","volume":"17","author":[{"family":"Baur","given":"David"},{"family":"Gladstone","given":"Beryl Primrose"},{"family":"Burkert","given":"Francesco"},{"family":"Carrara","given":"Elena"},{"family":"Foschi","given":"Federico"},{"family":"Döbele","given":"Stefanie"},{"family":"Tacconelli","given":"Evelina"}],"issued":{"date-parts":[["2017",9,1]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}].

Conclusions

Triazole fungicide use in agriculture has increased >4-fold during 2006–2016 in the United States, driven primarily by increases in propiconazole and tebuconazole, with the largest increases in central parts of the United States. Exposure of *A. fumigatus* to fungicides can select for mutations that cause resistance to the primary antifungals used to treat human aspergillosis. Data on agricultural triazole use can inform further research, risk assessments, and policy decisions related to resistant fungal infections associated with patient illness and death.

Acknowledgments

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Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention (CDC).

References

[ADDIN ZOTERO_BIBL {"uncited":[],"omitted":[],"custom":[]} CSL_BIBLIOGRAPHY]

Figures

Figure 1: Triazole use by year in metric tons (graph)
Figure 2: Triazole use by A. crop type and B. compound type in metric tons (graph)
Figure 3: Triazole use by state in metric tons (map)

Supplemental tables

Supplemental Table 1: Triazole use by year and crop type in metric tons
Supplemental Table 2: Triazole use by year and compound type in metric tons
Supplemental Table 3: Triazole use by state in metric tons (differences and % change)

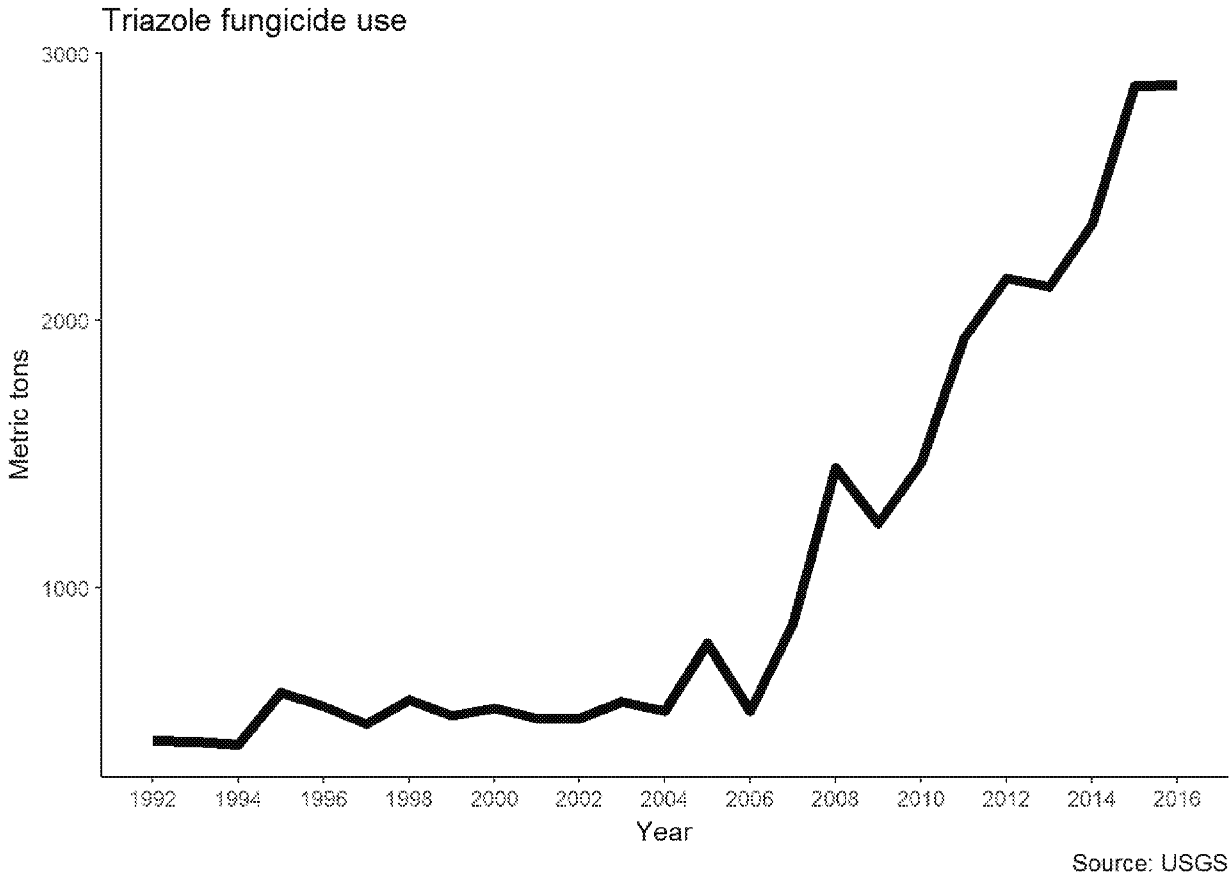
Supplemental figure

Supplemental Figure: Triazole use by state and crop type (in metric tons)

Supplemental data

Excel file: Triazole use by state by year

Figure 1: Triazole use by year (in metric tons), 1992–2016

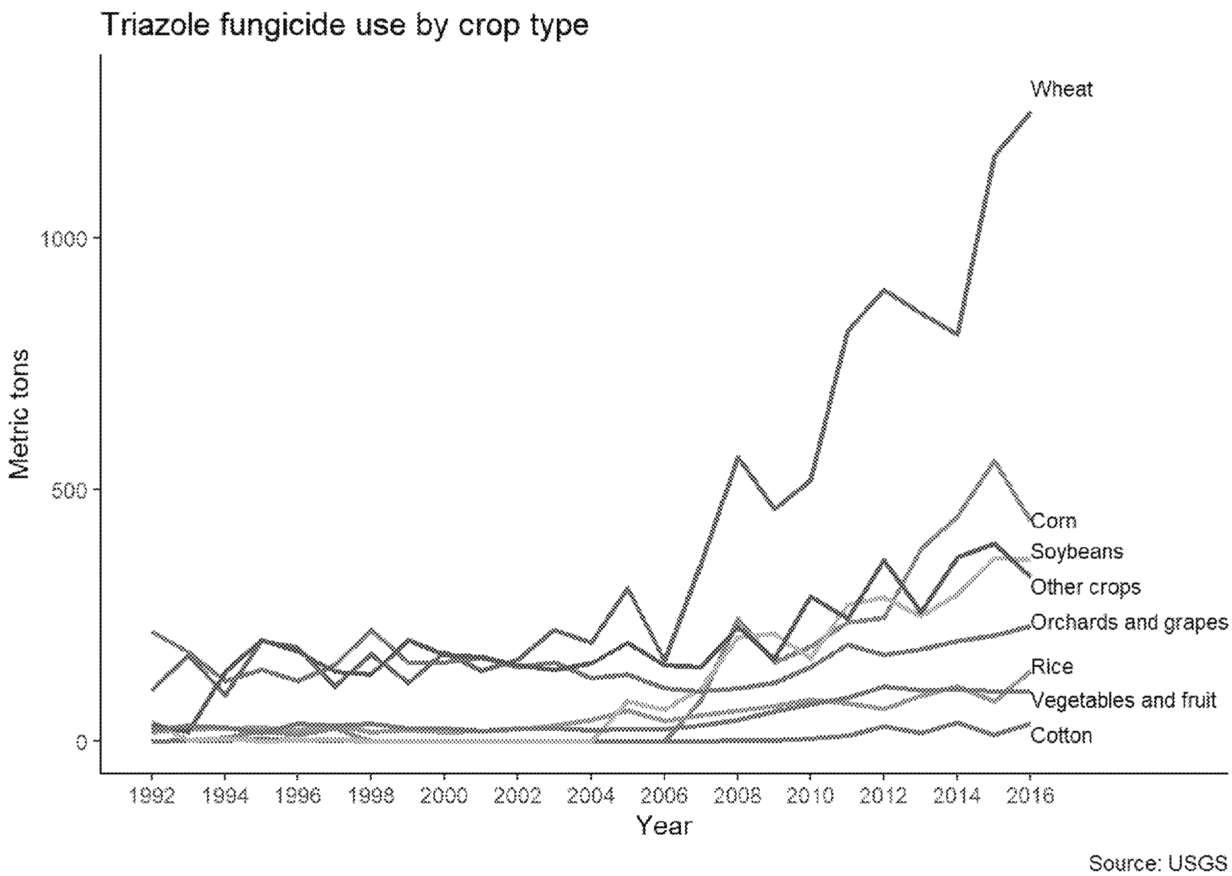


Calculated the average triazole fungicide use by taking the average of USGS' low and high annual agricultural pesticide estimates.

USGS: United States Geological Survey

Figure 2: Triazole use by crop and compound type (in metric tons), 1992–2016

A. By crop type (in metric tons), 1992–2016

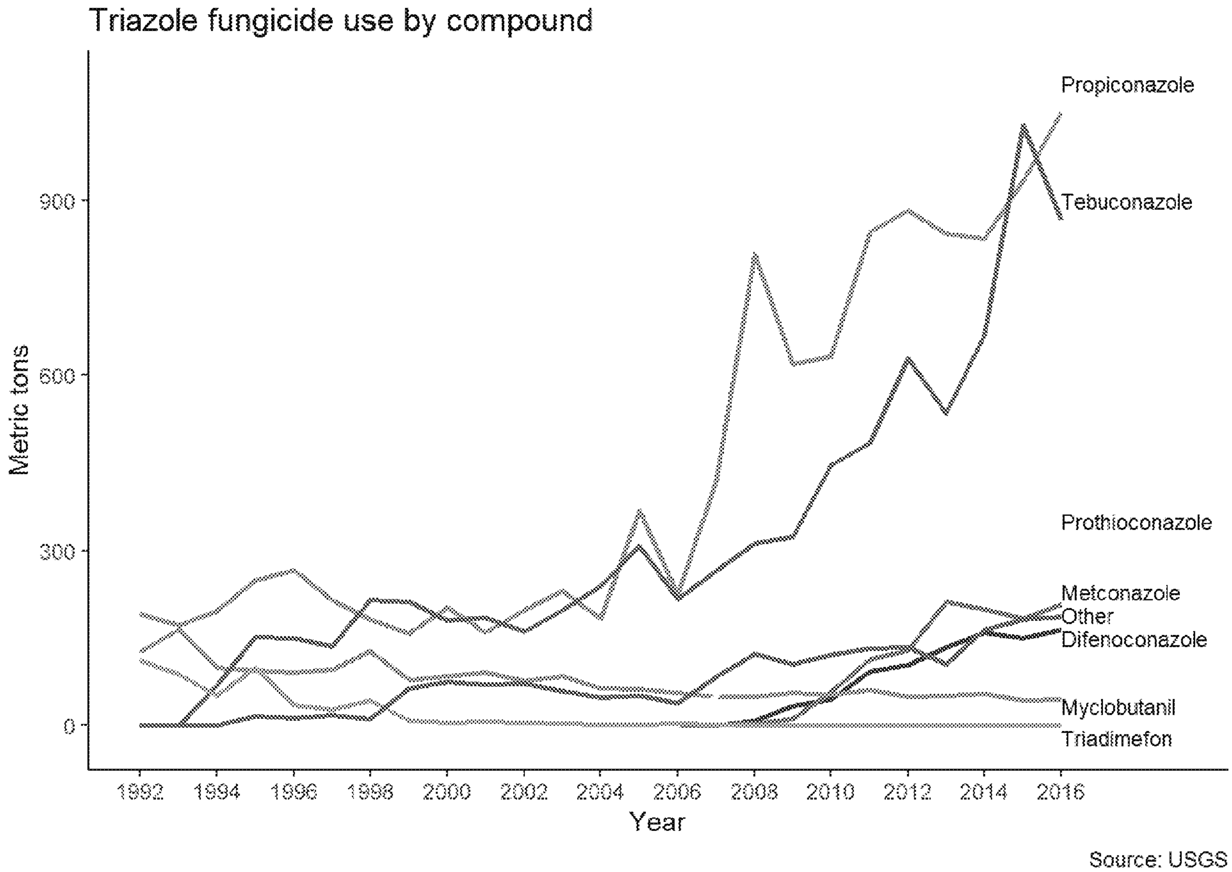


Crops were grouped into 8 categories:

1. Corn
2. Cotton
3. Orchards and grapes (stone fruit trees, citrus, nut trees, apples, pears, and grapevines)
4. Other crops
5. Rice
6. Soybeans
7. Vegetables and fruit (all vegetables and non-orchard fruit, including beans, peas, greens, berries, and melons)
8. Wheat

The following crop combinations were grouped into other crop type category: Pasture and Hay (cropland for pasture, fallow and idle cropland, pastureland, and other hay); Alfalfa; and Other (sorghum, non-wheat grains, tobacco, peanuts, sugarcane, sugar beets, and other miscellaneous crops).

B. Triazole use by compound type (in metric tons), 1992–2016



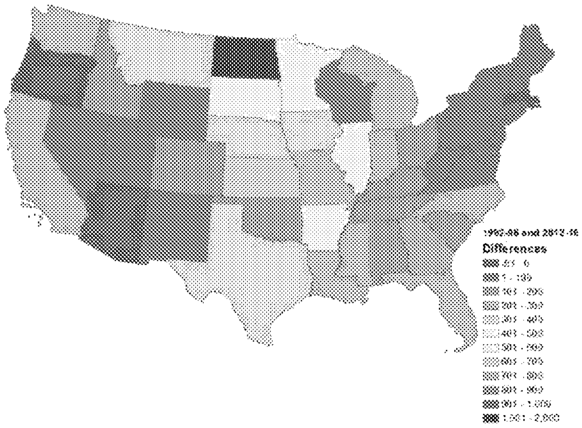
All 16 triazoles included in the USGS dataset were grouped into 8 triazole categories:

1. Difenoconazole
2. Metconazole
3. Myclobutanil
4. Other
5. Propiconazole
6. Prothiconazole
7. Tebuconazole
8. Triadimefon

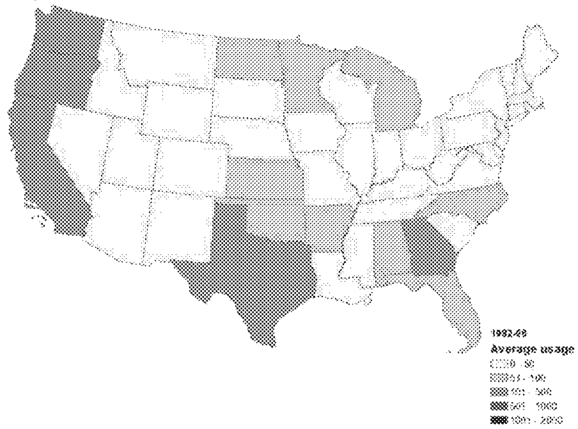
The following triazoles were grouped into other triazole compound type category: cyproconazole, fenbuconazole, flusilazole, flutriafol, ipconazole, tetraconazole, triadimenol, triticonazole, and uniconazole.

Figure 3: Triazole fungicide usage map by state, 1992–2016

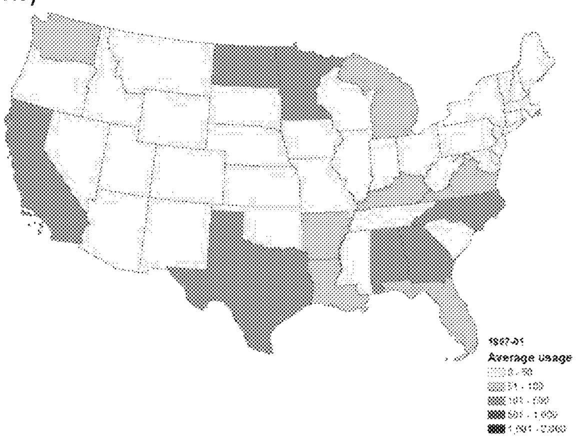
A: Differences in triazole fungicide usage 2012–16 and 1992–96 (in metric tons)



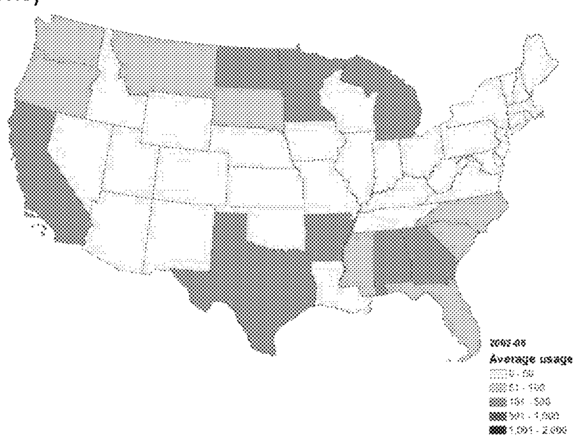
B: Triazole fungicide usage 1992–1996 (in metric tons)



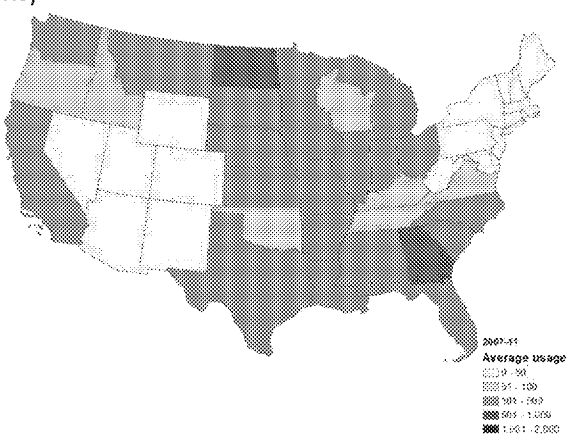
C: Triazole fungicide usage 1997–2001 (in metric tons)



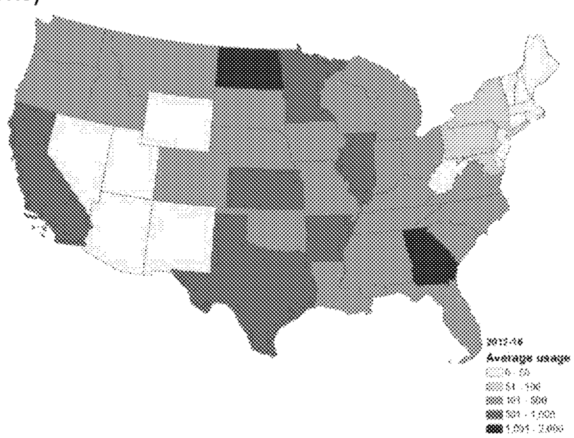
D: Triazole fungicide usage 2002–2006 (in metric tons)



E: Triazole fungicide usage 2007–2011 (in metric tons)



F: Triazole fungicide usage 2012–2016 (in metric tons)



Supplemental Table 1: Triazole use by year and crop type in metric tons, 1992–2016

Year	Wheat	Corn	Soybeans	Other crops	Orchards	Rice	Vegetables	Cotton	Total
1992	100	0	40	32	217	18	21	0	428
1993	171	1	2	19	177	23	30	2	425
1994	92	7	4	138	120	25	28	1	415
1995	198	20	0	200	142	28	16	4	608
1996	185	12	1	179	119	21	35	2	554
1997	108	26	4	138	150	31	32	2	490
1998	173	0	0	131	221	18	34	0	578
1999	115	0	0	200	157	22	26	0	520
2000	176	0	0	171	156	18	26	0	547
2001	139	0	0	167	165	20	19	0	511
2002	162	0	0	152	148	24	25	0	511
2003	221	0	0	142	156	30	25	0	573
2004	194	0	0	154	126	42	22	0	537
2005	304	0	78	194	132	59	23	0	792
2006	160	0	61	151	104	40	23	0	539
2007	352	82	105	147	99	50	31	0	866
2008	564	243	206	227	104	60	43	1	1447
2009	461	156	213	164	116	69	59	1	1240
2010	519	187	164	287	147	83	74	5	1466
2011	816	236	272	243	192	75	86	10	1930
2012	897	245	286	359	172	64	108	28	2158
2013	851	381	248	257	182	90	101	15	2125
2014	808	447	291	365	199	108	102	37	2358
2015	1164	556	364	394	211	79	99	12	2878
2016	1253	437	361	327	229	140	99	35	2880

Crops were grouped into 8 categories: 1. Corn; 2. Cotton; 3. Orchards and grapes (stone fruit trees, citrus, nut trees, apples, pears, and grapevines); 4. Other crops; 5. Rice; 6. Soybeans; 7. Vegetables and fruit (all vegetables and non-orchard fruit, including beans, peas, greens, berries, and melons); and 8. Wheat. The following crop combinations were grouped into other crop type category: Pasture and Hay (cropland for pasture, fallow and idle cropland, pastureland, and other hay); Alfalfa; and Other (sorghum, non-wheat grains, tobacco, peanuts, sugarcane, sugar beets, and other miscellaneous crops).

Supplemental Table 2: Triazole use by year and compound type in metric tons, 1992–2016

Year	Propiconazole	Tebuconazole	Prothioconazole	Metconazole	Difenoconazole	Myclobutanil	Triadimefon	Other
1992	191	0	0	0	0	126	112	0
1993	172	0	0	0	0	164	88	0
1994	196	68	0	0	0	99	51	0
1995	248	151	0	0	0	94	99	16
1996	265	149	0	0	0	91	35	13
1997	215	135	0	0	0	95	28	18
1998	182	215	0	0	0	127	42	12
1999	158	211	0	0	0	79	8	64
2000	202	180	0	0	0	84	6	75
2001	159	185	0	0	0	90	7	70
2002	198	160	0	0	0	76	5	72
2003	231	197	0	0	0	84	3	59
2004	184	240	0	0	0	63	2	49
2005	368	307	0	0	0	63	2	51
2006	225	217	0	0	0	56	3	38
2007	416	265	49	2	0	50	2	83
2008	807	312	146	2	8	49	0	122
2009	619	324	91	11	33	55	0	106
2010	631	446	113	58	44	52	0	121
2011	845	485	203	113	92	61	0	132
2012	882	629	230	129	104	50	0	134
2013	842	535	248	211	133	50	0	106
2014	833	668	279	200	159	53	0	164
2015	933	1028	357	184	150	44	0	182
2016	1050	866	361	207	164	45	0	186

All 16 triazoles included in the USGS dataset were grouped into 8 triazole categories: 1. Difenoconazole; 2. Metconazole; 3. Myclobutanil; 4. Other; 5. Propiconazole; 6. Prothiconazole; 7. Tebuconazole; and 8. Triadimefon. The following triazoles were grouped into other triazole compound type category: cyproconazole, fenbuconazole, flusilazole, flutriafol, ipconazole, tetraconazole, triadimenol, triticonazole, and uniconazole.

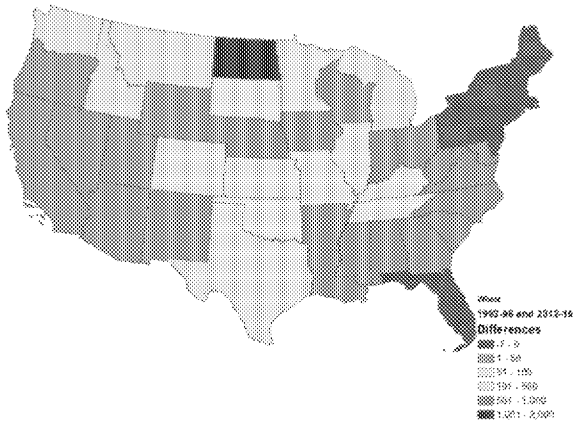
Supplemental Table 3: Triazole use by state (in metric tons), 1992–2016

State	Differences during 2012–2016 and 1992–1996		Percent change between 1992–1996 and 2012–2016		Sum of use during 1992–1996		Sum of use during 2012–2016	
	Order	Metric tons	Order	Percent change	Order	Metric tons	Order	Metric tons
North Dakota	1	1731	2	2517%	10	69	1	1800
Georgia	2	719	26	249%	2	289	2	1008
Kansas	3	621	12	828%	8	75	4	696
Minnesota	4	581	7	987%	12	59	5	640
Illinois	5	505	3	2309%	28	22	6	527
South Dakota	6	432	46	N/A	44	0	9	432
Arkansas	7	424	21	431%	6	98	8	522
Texas	8	390	23	291%	4	134	7	523
Iowa	9	387	1	5345%	34	7	11	394
Nebraska	10	377	6	1179%	19	32	10	409
Montana	11	312	9	886%	17	35	12	347
Michigan	12	258	18	484%	13	53	14	312
Indiana	13	256	4	1585%	32	16	18	272
Louisiana	14	253	13	708%	16	36	16	289
Missouri	15	242	5	1225%	29	20	20	262
Florida	16	233	21	447%	14	52	17	285
California	17	230	39	48%	1	480	3	711
North Carolina	18	229	23	315%	9	73	15	301
Washington	19	220	26	174%	5	127	12	347
Mississippi	20	212	9	856%	24	25	21	237
Alabama	21	185	26	213%	7	87	18	272
South Carolina	22	161	16	596%	22	27	23	188
Kentucky	23	158	13	675%	25	23	24	182
Ohio	24	152	13	650%	25	23	26	176
Tennessee	25	150	18	511%	20	29	25	179
Idaho	26	146	16	568%	23	26	27	172

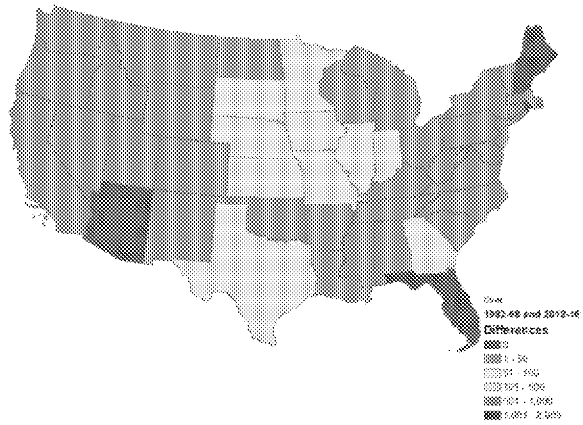
State	Differences during 2012–2016 and 1992–1996		Percent change between 1992–1996 and 2012–2016		Sum of use during 1992–1996		Sum of use during 2012–2016	
	Order	Metric tons	Order	Percent change	Order	Metric tons	Order	Metric tons
Oklahoma	27	144	26	235%	11	61	22	206
Colorado	28	126	18	539%	25	23	28	149
Wisconsin	29	94	8	961%	33	10	30	104
Virginia	30	58	33	128%	15	45	31	103
Maryland	31	35	26	178%	29	20	32	55
Pennsylvania	32	23	33	83%	21	28	34	51
New York	33	18	33	54%	18	34	33	52
Wyoming	34	11	46	N/A	44	0	38	11
New Mexico	35	9	26	184%	37	5	36	14
New Jersey	36	7	33	140%	37	5	37	12
Maine	37	6	23	311%	40	2	39	8
Delaware	38	2	39	12%	31	19	35	21
Nevada	38	2	9	868%	44	0	43	2
Utah	40	1	39	40%	36	4	39	5
West Virginia	40	1	39	23%	39	6	41	8
Vermont	40	1	26	178%	42	1	43	2
Connecticut	40	1	33	114%	42	1	46	1
New Hampshire	40	1	46	N/A	44	0	46	1
Massachusetts	45	0	39	81%	40	0	43	0
Rhode Island	45	0	33	-2%	44	2	48	2
Arizona	47	-3	39	-48%	34	7	42	4
Oregon	48	-81	39	-43%	3	191	29	109

Supplemental Figure: Triazole fungicide usage by state and crop type, 1992–2016

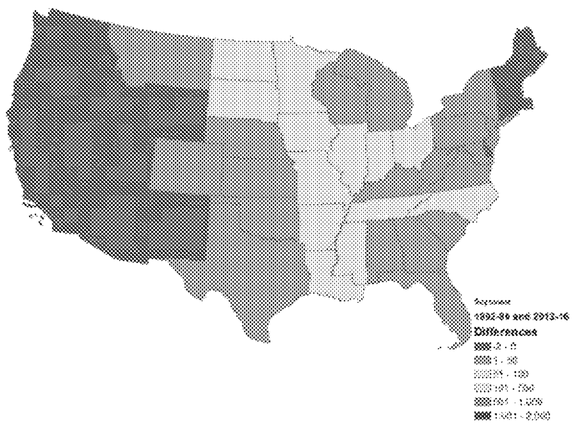
A: Differences in triazole fungicide usage for wheat 2012–16 and 1992–96 (in metric tons)



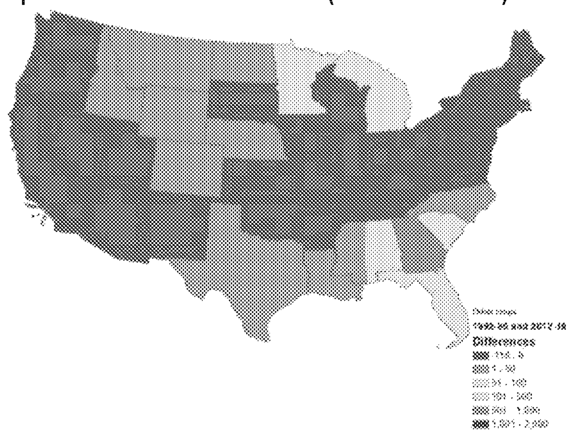
B: Differences in triazole fungicide usage for corn 2012–16 and 1992–96 (in metric tons)



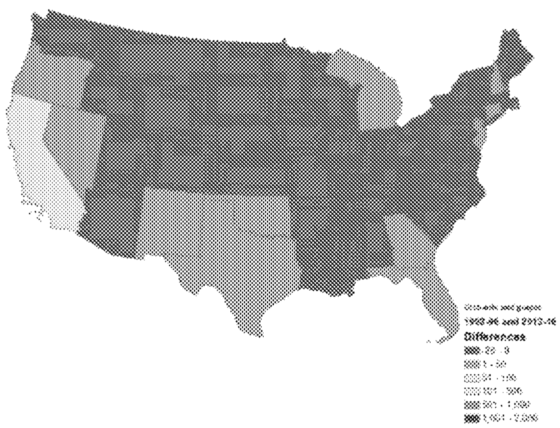
C: Differences in triazole fungicide usage for soybeans 2012–16 and 1992–96 (in metric tons)



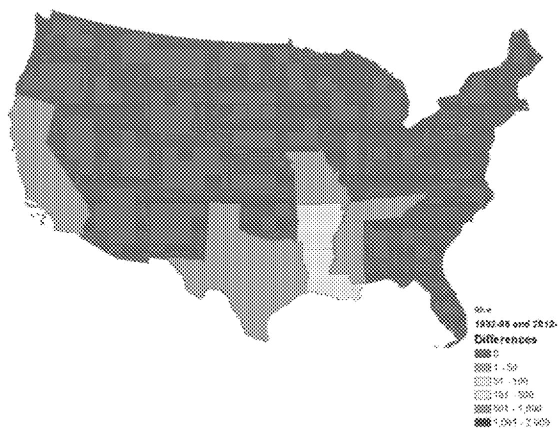
D: Differences in triazole fungicide usage for other crops 2012–16 and 1992–96 (in metric tons)



E: Differences in triazole fungicide usage for orchards and grapes 2012–16 and 1992–96 (in metric tons)

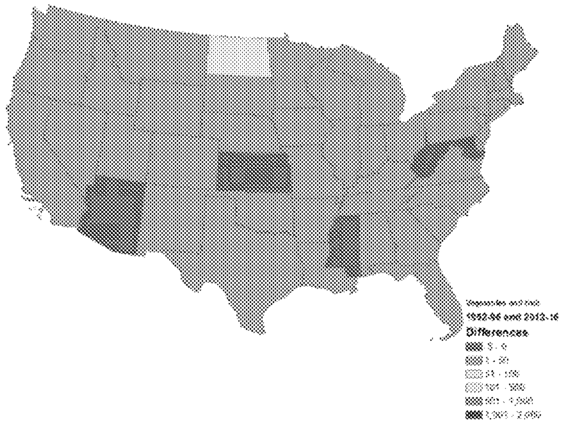


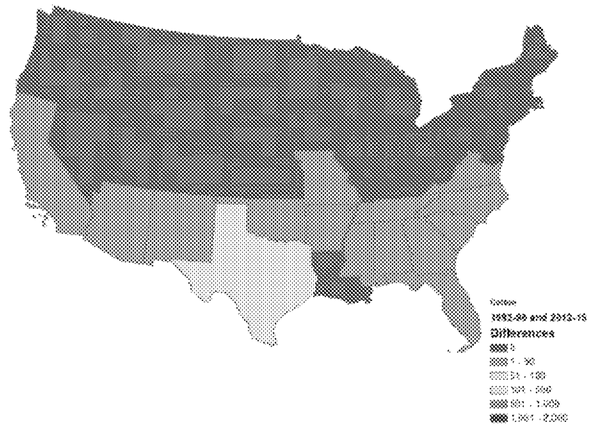
F: Differences in triazole fungicide usage for rice 2012–16 and 1992–96 (in metric tons)



G: Differences in triazole fungicide usage for vegetables and fruit 2012–16 and 1992–96 (in metric tons)

H: Differences in triazole fungicide usage for cotton 2012–16 and 1992–96 (in metric tons)





Crops were grouped into 8 categories: 1. Corn; 2. Cotton; 3. Orchards and grapes (stone fruit trees, citrus, nut trees, apples, pears, and grapevines); 4. Other crops; 5. Rice; 6. Soybeans; 7. Vegetables and fruit (all vegetables and non-orchard fruit, including beans, peas, greens, berries, and melons); and 8. Wheat. The following crop combinations were grouped into other crop type category: Pasture and Hay (cropland for pasture, fallow and idle cropland, pastureland, and other hay); Alfalfa; and Other (sorghum, non-wheat grains, tobacco, peanuts, sugarcane, sugar beets, and other miscellaneous crops).